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Dear sponsors & exhibitors,

We would like to welcome you to the 10th Joint Annual Meeting of the International Cytokine Society (ICS) and the International Society for Interferon and Cytokine Research (ISICR), which will take place in Geneva in 2012. The objective of the Meeting is to promote interactions between scientists performing cutting-edge studies of the molecular mechanisms of cytokine function, signal transduction, and gene expression, and those working in drug discovery and in the clinic to translate this knowledge into novel therapies for human diseases. The therapeutic potential of targeting cytokines and of modulating their signaling pathways enforce the need for enhanced interactions between basic, translational, and clinical researchers. For this purpose, the sessions will include presentations of cutting-edge basic science and clinical science both in plenary and concurrent sessions.

The broad themes proposed will incorporate basic and clinical research on innate immunity, host-pathogen interactions, inflammation, autoimmunity, cell signaling, transcriptional and post-transcriptional gene regulation and tumor immunity. These themes are consistent with the well-recognized strengths of many of the world-class research institutions in Switzerland, as well as the research interests of both the ICS and ISICR societies. In addition, we have also included sessions focused on osteoimmunology, tissue repair, and the link between inflammation and metabolism.

In addition to this exciting scientific program, we hope that you will take the time to visit Geneva and its beautiful countryside. Geneva is a lively international city located in the middle of Europe close to the Swiss and French Alps with many possibilities for pleasant day trips for those who wish to extend their stay after the Meeting.

On behalf of all participants, we would like to express our gratitude to you! The dissemination of knowledge that takes place at such meetings and the interactions and collaborations that are established are essential for future advances in biomedical research. Successful meetings simply would not be possible without substantial support from corporations, foundations, and institutes such as yours.

We look forward to seeing you in what promises to be an exciting and timely Meeting.

Cem Gabay  
Chairman

Amanda Proudfoot  
Co-Chair
## Committee Members

### Meeting Chairs

<table>
<thead>
<tr>
<th>Name</th>
<th>Location</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cem Gabay</td>
<td>Geneva</td>
</tr>
<tr>
<td>Amanda Proudfoot</td>
<td>Geneva</td>
</tr>
</tbody>
</table>

### Local Organizing Committee

<table>
<thead>
<tr>
<th>Name</th>
<th>Location</th>
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</thead>
<tbody>
<tr>
<td>Cem Gabay</td>
<td>Geneva</td>
</tr>
<tr>
<td>Amanda Proudfoot</td>
<td>Geneva</td>
</tr>
<tr>
<td>Walter Reith</td>
<td>Geneva</td>
</tr>
</tbody>
</table>

### ICS-ISICR Scientific Committee

<table>
<thead>
<tr>
<th>Name</th>
<th>Location</th>
</tr>
</thead>
<tbody>
<tr>
<td>Luke O’Neill</td>
<td>Dublin – Ireland</td>
</tr>
<tr>
<td>Eleanor Fish</td>
<td>Toronto – Canada</td>
</tr>
<tr>
<td>Chris Hunter</td>
<td>Philadelphia – USA</td>
</tr>
<tr>
<td>Ganes Sen</td>
<td>Cleveland – USA</td>
</tr>
<tr>
<td>George Stark</td>
<td>Cleveland – USA</td>
</tr>
<tr>
<td>Dr. Jun-Ichiro Inoue</td>
<td>Tokyo – Japan</td>
</tr>
<tr>
<td>Hans Acha-Orbea</td>
<td>Lausanne</td>
</tr>
<tr>
<td>Federica Sallusto</td>
<td>Bellinzona</td>
</tr>
<tr>
<td>Manfred Kopf</td>
<td>Zürich</td>
</tr>
<tr>
<td>Marie Kosco-Vilbois</td>
<td>Geneva</td>
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### Scientific Programme Information

<table>
<thead>
<tr>
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<tbody>
<tr>
<td><strong>08.30-10.30</strong></td>
<td><strong>08.00-10.00</strong></td>
<td><strong>08.30-10.30</strong></td>
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<tr>
<td>Plenary Session</td>
<td>Plenary Session</td>
<td>Plenary Session</td>
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<tr>
<td>Cytokines and infectious diseases: <em>host-pathogen interactions</em></td>
<td>Interferons in innate immunity</td>
<td><em>T-cell subsets and cytokines</em></td>
<td></td>
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<tr>
<td><strong>10.30-11.00</strong></td>
<td><strong>10.00-10.30</strong></td>
<td><strong>10.30-11.00</strong></td>
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<tr>
<td>Coffee Break</td>
<td>Coffee Break</td>
<td>Coffee Break</td>
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<tr>
<td><strong>11.00-12.30</strong></td>
<td><strong>10.30-12.00</strong></td>
<td><strong>11.00-12.30</strong></td>
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<tr>
<td>Disease Oriented Symposium 1, 2</td>
<td>Disease Oriented Symposium 3, 4</td>
<td>Disease Oriented Symposium 5, 6</td>
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<tr>
<td>1. Cytokines regulation of tissue remodelling</td>
<td>3. IL-17 and related cytokines in inflammatory diseases</td>
<td>5. Cytokines targeting in autoimmune diseases I</td>
<td></td>
</tr>
<tr>
<td>2. IFN treatment of viral infections</td>
<td>4. IL-6 and inflammatory diseases</td>
<td>6. Cytokines targeting in autoimmune diseases II</td>
<td></td>
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<tr>
<td><strong>12.30-14.00</strong></td>
<td><strong>12.00-14.00</strong></td>
<td><strong>12.30-14.00</strong></td>
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<tr>
<td>Lunch Break</td>
<td>Poster Session 2</td>
<td>Lunch Break</td>
<td></td>
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<tr>
<td><strong>14.00-16.00</strong></td>
<td><strong>14.00-16.00</strong></td>
<td><strong>14.00-16.00</strong></td>
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<tr>
<td>Parallel Session 1, 2</td>
<td>Parallel Session 3, 4</td>
<td>Parallel Session 7, 8</td>
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<tr>
<td><strong>16.00-18.30</strong></td>
<td><strong>16.00-16.30</strong></td>
<td><strong>16.00-16.30</strong></td>
<td><strong>16.00-16.00</strong></td>
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<tr>
<td>Poster Session 1</td>
<td>Coffee Break</td>
<td>Closing Ceremony</td>
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<tr>
<td>Wine &amp; Cheese</td>
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<tr>
<td><strong>18.00-19.00</strong></td>
<td><strong>16.30-18.30</strong></td>
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<tr>
<td>Award Presentation</td>
<td>Parallel Session 5, 6</td>
<td></td>
<td></td>
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<tr>
<td><strong>19.00-20.30</strong></td>
<td>5. MicroRNA and gene regulation</td>
<td></td>
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<tr>
<td>Honorary and Keynote</td>
<td>6. IFN regulation of viral pathogenesis</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>20.30-21.30</strong></td>
<td><strong>20.00-00.00</strong></td>
<td>Meeting Dinner</td>
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<tr>
<td>Welcome Reception</td>
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</tbody>
</table>

**Sponsorship Opportunities:** (more details on page 9)
THE MEETING VENUE (CICG) & GENEVA

About Geneva

In a country of spectacular natural beauty, Geneva is one of Switzerland’s most beautiful cities. Set on the banks of Lake Leman between the Alps and the Jura mountains in the Southwest corner of Switzerland, Geneva enjoys a temperate climate and breath taking scenery, with Alpine lakes, snow-capped mountains, lush forests and enchanting countryside.

As host city of many International Organizations, Geneva has a long tradition of humanism and communication as well as an established reputation as a centre for arts and culture. The city has over 30 museums, as well as many art galleries, theatres and an opera house. Fashionable hotels, chic restaurants and elegant shops jostle for position along the flower-decked lakefront that encircles the famous “Jet d’Eau” Fountain. The fountain shoots 500 litres of water per second to a height of 140 meters above the Lake Geneva. The “Jet d’Eau” has come to symbolize Geneva around the world and traditionally signals the coming of spring each year.

Transportation and Accommodation

Located in the centre of Europe, Geneva is easily accessible by air from all major European cities, and there are interesting low-budget connections from several of them. The international airport is only 15 minutes from the City Centre. Geneva has a wide choice of hotels in different categories!

Getting Around

Geneva’s centre is small enough to walk around. For the suburbs you’ll need to take a tram or bus. Daily passes are provided free of charge to Geneva hotel guests, courtesy of the Geneva Tourist Board.

Sights

The river Rhône, boasting several bridges amongst which the historical Pont de l’Ile, flows out of Lac Leman to bisect Geneva’s bustling centre. The Rive Gauche, south of the river, is the most active part of Geneva with a grid of waterfront streets comprising the main shopping and business districts. Behind, winding up the hillside, are the narrow, cobbled lanes of the old town, whose principal thoroughfare, Grand Rue, leads to the seventeenth-century Hotel de Ville and the arcaded armoury, backed by a lovely terrace. The streets around the Hotel de Ville, with their quaint cafés, second-hand bookshops and art galleries, make for a pleasant stroll of discovery. Nearby is the Romanesque cathedral, a monumental edifice whose austere, lofty interior contains the beautiful, fifteenth-century frescoes of the Chappelle des Maccabées. Among the city’s several museums, the pick is the Musée d’Art et d’Histoire (2, rue Charles-Galland), which holds a massive archaeological collection, a selection of Swiss landscape painters and a magnificent altar-piece carved for the cathedral in the 1440’s and moved here for safe-keeping. Finally, make a point of seeing Geneva’s pride and joy, the dramatic 140-metre high Jet d’Eau, spouting high above the ornamental flowerbeds of the surrounding Jardin Anglais, down by the lake.
Meeting Venue

The Cytokines 2012 Meeting will take place at the International Conference Centre Geneva (CICG) located near the United Nations and only 10 minutes from City Centre.

The CICG Conference Centre

Rue de Varembé 17 – CP 13
CH-1211 Geneva 20
Switzerland
Phone: +41 (0)22 791 91 11
Fax: +41 (0)22 791 90 64
Internet: www.cicg.ch

Organizing Secretariat

Cytokines 2012
c/o MCI Suisse SA
Rue de Lyon 75
P.O. Box 502
1211 Geneva 13
Switzerland
Website: www.cytokines2012.com

Exhibition & Sponsoring Queries:
Tel.: +41 22 33 99 650
Fax: +41 22 33 99 601
Email: christopher.day@mci-group.com

Registration & Accommodation Queries:
Tel.: +41 22 33 99 574
Fax: +41 22 33 99 631
Email: cytokines2012reg@mci-group.com

IMPORTANT DATES & DEADLINES

<table>
<thead>
<tr>
<th>Event</th>
<th>Date</th>
</tr>
</thead>
<tbody>
<tr>
<td>Opening of registration</td>
<td>15 February 2012</td>
</tr>
<tr>
<td>Opening of abstract submission</td>
<td>15 February 2012</td>
</tr>
<tr>
<td>Abstract submission deadline</td>
<td>11 May 2012, midnight CET</td>
</tr>
<tr>
<td>Early registration deadline</td>
<td>11 May 2012</td>
</tr>
</tbody>
</table>
SPONSORSHIP RECOGNITION LEVEL/CATEGORY

Sponsorship recognition level is calculated based on the total amount of your company’s investment in Cytokines 2012 (to include exhibition space and sponsorship opportunities as listed herein).

Depending on your total level of investment in Cytokines 2012, your company’s support will be acknowledged and recognised on the Meeting website www.Cytokines2012.com at the following different levels:

<table>
<thead>
<tr>
<th>Sponsorship Level</th>
<th>Total Contribution</th>
<th>Complimentary Delegate Registrations</th>
<th>Invitations to the Meeting Dinner</th>
</tr>
</thead>
<tbody>
<tr>
<td>Platinum</td>
<td>From CHF 50’000</td>
<td>4</td>
<td>3</td>
</tr>
<tr>
<td>Gold</td>
<td>From CHF 40’000</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>Silver</td>
<td>From CHF 30’000</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Bronze</td>
<td>From CHF 20’000</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

* Total contributions include total payments for all sponsorship opportunities listed herein. This amount does not include food, beverage and conference registration fees for organisation staff.

Sponsorship level recognition including company logo on all Meeting documents, signage and website (www.cytokines2012.com).
The Exhibition is an integral part of the Cytokines 10th Joint Annual Meeting. As an exhibitor, you will enjoy prime exposure and direct marketing opportunities with the key players and decision makers in the field. The Floor Plan is designed to maximize the exhibitor’s exposure to the delegates.

All Coffee Breaks and complimentary lunches will be served in the exhibition area. Particular attention will be given to signage and decoration enabling an easy and convenient visit of the exhibition.

Exhibitor’s profiles will be listed in the Final Programme and on Cytokines 2012 website.

### Table Top

- 4 sq. meter of floor space
- 1 table (L 150cm x W 75cm / H 80cm)
- 2 chairs
- 1 electrical plug (10A, 230V, 2.3kW)
- Limit to one table top per company/association.

### Space Only

Minimum space 6 Sq. meter = CHF 3’300.-

Benefits include:
- Exhibition space
- 2 Exhibitor badges per 6 sqm space, 1 additional badge for each additional 3 sqm booked
- Coffee Breaks and Welcome Reception

Exhibitors occupying space-only stands are required to submit a detailed plan of their stand to The Organizers for approval by 30 June 2012.

### Shell Scheme Package

Minimum space 6 sq. meter = CHF 900.- as a supplement to space only

Benefits include:
- Exhibition space
- Lighting (3 spots pr 6 sq.m. space)
- Electrical supply
- Carpeting, 1 table and 2 chairs per 6 sq.m. space
- Fascia board for company name
- 2 Exhibitor badges per 6 sqm space, 1 additional badge for each additional 3 sqm booked
- Coffee Breaks and Welcome Reception
- Daily stand cleaning

All additional equipment (such as structure, walls, electricity, decoration, carpet, furniture, etc...) will be ordered and paid separately by the sponsor/exhibitor.

* Prices excl. Swiss VAT 8%
SPONSORSHIP OPPORTUNITIES

How do I become a sponsor?

In the following pages, we are offering your company the opportunity to become a sponsor of the Cytokines 10th Joint Annual Meeting 2012, enabling you to promote your scientific endeavours to a targeted audience.

You can contribute in 2 ways:

- Purchase exhibition space
- Sponsor various items in the Meeting activities

The various sponsorship options are listed in the following pages in which you can select different elements up to the amount of the financial support you are planning.

All prices quoted are in Swiss Francs (CHF) and are exclusive of Swiss VAT 8%.

The application form enclosed at the end of this document should be duly completed and returned in order to confirm your participation at the Cytokines 2012 Meeting.

All sponsors will be treated on a "first come, first served" basis.

A. PUBLICATION / ADVERTISING

ADVERTISING IN THE MINI/POCKET PROGRAMME

All delegates will receive a pocket sized programme, inserted into their name badge holder. This programme will contain a summary of all information including session details, exhibition information and social events.

Benefits include:

- Full colour advertisement page (A6 Format) on the back cover page of the mini programme (Artwork to be supplied by sponsor)
- Exclusive sponsorship
- Acknowledgement in Final Programme with company logo

  - OUTSIDE BACK COVER: CHF 10’000*

ADVERTISING IN THE FINAL PROGRAMME

The Final Programme for Cytokines 2012 in Geneva is distributed to all delegates on-site inside the official Cytokines 2012 Meeting bag. The programme contains the final scientific programme, the social programme and all general information about the Meeting. The delegates will use the Final Programme to plan and organise their activities on a daily basis.

  - OUTSIDE BACK COVER: CHF 2’500*
  - INSIDE FRONT COVER: CHF 2’000*
  - INSIDE BACK COVER: CHF 2’000*
  - INSIDE PAGE: CHF 1’000*

* Prices excl. Swiss VAT 8%

1 please see Exhibition p.8
B. MEETING SERVICES

SPEAKER PREVIEW ROOM

The Sponsorship of this area where opinion leaders and faculty from around the world will converge to prepare for their presentations represents a unique opportunity to reach all speakers taking part in the Meeting. Benefits of this sponsorship include:

- Sponsors homepage and screen saver on all computer screens
- Opportunity to provide branded mouse pads (sponsor provides at sponsor cost)
- Sponsors logo on signage at speakers preview room
- Acknowledgement as a Meeting sponsor in the Final Programme

- **SPEAKER PREVIEW ROOM:** CHF 5’000*

INTERNET CORNER

The internet corner is one of the most visited points by all conference participants. At the Cytokines 2012 Meeting, it will offer at least 5 computer stations with free internet access.

- Sponsors homepage and screen saver on all computer screens
- Opportunity to provide branded mouse pads (sponsor provides at sponsor cost)
- Sponsors logo on signage at the Internet Corner
- Acknowledgement as a Meeting sponsor in the Final Programme

- **INTERNET CORNER:** CHF 5’000*

MEETING APPLICATION ON SMARTPHONE

Make the most of your onsite investment by promoting it first online!

Sponsoring the Meeting application on Smartphone is an innovative interactive service that you can provide to the Cytokines 2012 delegates. This technology will enable delegates to download the complete programme of Cytokines 2012 or even to read selected posters.

Benefits include:

- Cytokines Meeting information available to download from the Cytokines 2012 website
- Company logo on the welcome page of the application
- Acknowledgement in the Cytokines 2012 Final Programme
- Acknowledgement on the Cytokines 2012 website

- **MEETING APPLICATION ON SMARTPHONE:** CHF 5’000*

C. MEETING PROGRAMME

PLENARY SESSIONS

4 Plenary sessions will take place from Wednesday 12 September to Friday 14 September on Cytokines 2012. Each plenary session will last 2 hours and will be presented by Key Opinion Leaders in their specific field. Benefits include:

- Acknowledgement in the Final Programme and on the Meeting website.
- Company logo on screen at the beginning and at the end of the session, and on signage at the entrance to the room

- **PLENARY SESSIONS:** CHF 20’000* per session

* Prices excl. Swiss VAT 8%
6 Disease Oriented Symposia will take place from Wednesday 12 September to Friday 14 September on Cytokines 2012. Each one will last 1.5 hour and will be presented by Key Opinion Leaders in their specific field. Benefits include:

- Acknowledgement in the Final Programme and on the Meeting website.
- Company logo on screen at the beginning and at the end of the session, and on signage at the entrance to the room.

**Symposium 1 to 6**

**Disease Oriented Symposium**

1. Cytokines regulation of tissue remodelling
2. IFN treatment of viral infections
3. IL-17 and related cytokines in inflammatory diseases
4. IL-6 and inflammatory diseases
5. Cytokines targeting in autoimmune diseases I
6. Cytokines targeting in autoimmune diseases II

**CHF 17’500.- per session**

**POSTER SESSIONS**

2 Poster Sessions will take place on Wednesday 12 September and Thursday 13 September on Cytokines 2012. Benefits include:

- Acknowledgement in the Final Programme and on the Meeting website
- Company logo on signage at the poster area
- Wine and Cheese provided by the Meeting on Wednesday 12 September (16:00-18:30)
- Lunch provided by the Meeting on Thursday 13 September (12:00-14:00)

**CHF 10’000**

**D. PROMOTIONAL MATERIAL**

**MEETING BAGS**

Sponsorship of the Meeting bags allows promoting your company and product in a very prominent and visual way. The logo of the sponsoring company will be printed on each Meeting bag along with the Cytokines 2012 logo. *Type, size and colour of the bags as well as position and size of the logo will be at the organiser’s discretion.*

Benefits include:

- One promotional leaflet (A4, double-sided) in the Meeting bags (to be provided by sponsor)
- Acknowledgement as a Meeting sponsor in the Final Programme

**CHF 20’000**

**WRITING PADS & PENS**

Your company logo will appear on the writing pads or pens. Delegates particularly appreciate the writing pads and pens as they are particularly useful during the scientific sessions. *Pads and Pens to be supplied by sponsors.*

Benefits include:

- Acknowledgement as a Meeting sponsor in the Final Programme
- Both the Pads and Pens will be inserted in to the Delegate Bags

**CHF 3’500**

**CHF 2’500**

**CHF 5’500**

*Prices excl. Swiss VAT 8%
LANYARDS

Your company logo will be screen-printed on the lanyards along with the Cytokines 2012 Meeting logo. This is one of the most prominent and visual sponsorship items as delegate wear them around their neck.

Benefits include:
- Logo printed on every delegates lanyard
- Acknowledgement as a Meeting sponsor in the Final Programme

- **LANYARDS**  
  **CHF 10’000***

INSERTS IN MEETING BAGS

Limited to 5 inserts maximum

The opportunity is offered to sponsors to provide an insert: A4 size double-sided, colour or black and white promotional piece. This flyer could be text only promoting activities on your exhibition stand or it may be an existing corporate flyer on information on your services or products. Company must supply an electronic (pdf, jpg, etc.) copy of insert to MCI Suisse prior to the meeting.

Benefits include:
- Acknowledgement in the Final Programme

- **PER INSERT**  
  **CHF 2’500***

E. SOCIAL EVENT SPONSORSHIP OPPORTUNITY

COFFEE BREAKS

Coffee Breaks, accessible to all delegates and accompanying persons, will be held in the exhibition area.

Benefits include:
- Acknowledgement as a Meeting sponsor in the Final Programme
- Signage featuring sponsor’s name and logo will be displayed on catering tables

- **COFFEE BREAKS (each)**  
  **CHF 5’000***

* Prices excl. Swiss VAT 8%

PAYMENT AND CANCELLATION CONDITIONS

By Credit Card

Visa, MasterCard and American Express are accepted.

Please request a Credit Card Authorisation Form from MCI Suisse SA: christopher.day@mci-group.com Payment will only be settled in Swiss Francs (CHF).

By Bank Transfer

Bank: UBS SA – 1211 Geneva 2 – Switzerland
Company: MCI Suisse SA for Cytokines 2012
Account N°: 369.393.00U
Clearing: 240
SWIFT: UBSWCHZH80A
IBAN: CH94 0024 0240 3693 9300 U

By Cheque

Cheque made in CHF, payable to MCI Suisse SA, Geneva.

Cancellation Policy

Cancellation and changes to your original booking must be made in writing to MCI.

For cancellation made:
- Until 31 March 2012 30% of the total cost of each item will be retained
- From 01 April 2012 50% of the total cost of each item will be retained
- From 01 June 2012 100% of the total cost of each item will be retained
RESERVATION
In order to be valid, your booth reservation must be completed on the APPLICATION FORM here enclosed and sent to MCI Suisse SA, along with a 50% deposit of the rental fee.

The signature on the booth reservation form and the deposit paid constitute a firm hire commitment and compel the subscriber to accept both the general conditions and the exhibition rules and regulations. No verbal or telephone agreement will commit MCI Suisse SA nor the Organizing Committee unless confirmed in writing.

The remaining subscription fee is to be settled by 31 May 2012 at the latest. Non-payment by this stated deadline will lead to the cancellation of your booth booking, without reimbursement of the deposit paid.

Site allocations will be attributed in reservation order of arrival and are subject to the full payment and the agreement of MCI Suisse SA and the Organizing Committee. Exhibitors will be requested to make choices, but neither Cytokines nor MCI Suisse SA can guarantee first choice. Once locations have been attributed, no change of location will be possible without MCI Suisse SA’s written agreement.

The Exhibition Floor Plan presented in this document is a non-contractual one. It is subject to acceptance by the Swiss Authorities and its official Fire & Safety Services. However, MCI Swiss reserves the right to change if deemed advisable, the location, importance and layout of the surfaces requested by the exhibitor. In the event of litigation, jurisdiction falls under the Geneva Law Courts alone.

TECHNICAL MANUAL
A Technical Manual will be sent to every registered exhibitor and sponsor by the end of May 2012. This manual will contain all information regarding general conditions, safety regulations, setting up of a booth and arrangement conditions, description of the booth, specification documents and maps along with order forms and prices for furniture, decoration and all necessary services (telephone, electricity, transport, storage...)

RULES AND REGULATIONS
MCI SUISSE SA has been entrusted with the general logistics and organisation of the Meeting and Exhibition of the Cytokines Annual Meeting 2012. It will be referred to as “The Organizers” here-below.

LOCAL AND SITE REGULATIONS
Exhibitors shall abide by the local and site regulations with respect to law and order, safe and security. The Organizers will take appropriate action against those who do not comply with the regulations.

The organizers have the authority to demand removal/change of any structure which is not in accordance with the Meeting rules or cancel participation. The decision of the organizers will be final and binding.

ENTRY TO THE EXHIBITION
Access to the exhibition will only be possible to registered conference participants or exhibition participants.

INSURANCE
The signatory renounces to take recourse against The Organizers or against the owners of the premises and undertakes to underwrite insurance policies covering all the risks incurred by the material exhibited (theft, damage, etc...) along with public liability covering the permanent or occasional staff employed by the company, present at the Meeting. In any case, the insurance protection will NOT be given to the exhibitors by The Organizers.

FORCE MAJEURE
In the event of force majeure, the exhibition dates may be changed or the latter may be purely and simply cancelled. In the last event, the disposable funds after payment of expenses will be shared between exhibitors in proportion to their payments without it being possible to take recourse against the organizer or the producer.

INTERPRETATION OF THE REGULATIONS AND AMENDMENTS
The MCI Suisse SA is the sole competent authority as to problems arising from the interpretation of the here enclosed regulations and their enforcement. Any expenses resulting from the non-observance of the here-enclosed regulations will be chargeable to the exhibitor.

MCI Suisse SA reserves the right to change or to complete the here-enclosed regulations at any time. If any changes should occur, all participating companies will be informed.

MCI Suisse SA
c/o Christopher Day
75 rue de Lyon
CH-1211 Geneva 13
Switzerland

Fax : +41 22 33 99 601
Email : christopher.day@mci-group.com
## EXHIBITION APPLICATION FORM AND CONTRACT

Please note that all acknowledgements of your company and listing of company name and address will be generated from the following information. It is mandatory to complete and sign this form to confirm your sponsorship. This form will be considered as a contract.

**PLEASE COMPLETE AND SEND TO:**
MCI Suisse SA – c/o Christopher Day – 75 rue de Lyon – CH-1211 Geneva 13 – Switzerland
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- [ ] **TABLE TOP CHF 3’000** (Limit 1 per exhibiting company)
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We require: ____________________________ sq. meters (minimum size 6 sq. meters)

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- All payments must be made in Swiss Francs (CHF)
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- For bookings made after 31 May 2012, the full amount is due at the time of reservation.

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**Signature and company stamp:**
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**Plenary Sessions** (exclusive)
- N°1- Wednesday 12 Sept. 2012, 08:30 – 10:30 CHF 20’000
- N°2- Thursday 13 Sept. 2012, 08:00 – 10:00 CHF 20’000
- N°3- Friday 14 Sept. 2012, 08:30 – 10:30 CHF 20’000

**Disease Oriented Symposium** (exclusive)
- N°1- Cytokines regulation of tissue remodelling Wednesday, 12 September 11:00 – 12:30 CHF 17’500
- N°2- IFN treatment of viral infections Wednesday, 12 September 11:00 – 12:30 CHF 17’500
- N°3- IL-17 and related cytokines in inflammatory diseases Thursday, 13 September 10:30 – 12:00 CHF 17’500
- N°4- IL-6 and inflammatory diseases Thursday, 13 September 10:30 – 12:00 CHF 17’500
- N°5- Cytokines targeting in autoimmune diseases I Friday, 14 September 11:00 – 12:30 CHF 12’500
- N°6- Cytokines targeting in autoimmune diseases II Friday, 14 September 11:00 – 12:30 CHF 12’500

**Poster Sessions** (exclusive)
- N°1- Wednesday 12 Sept. 2012, 16:00 – 18:30 CHF 10’000
- N°2- Thursday 13 Sept. 2012, 12:00 – 14:00 CHF 10’000

**PAYMENT SCHEDULE**
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From 01st June 2012
- 100% of the total cost of each item will be retained

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**Place and Date:**

**Signature and company stamp:**

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### Cytokines 2012 – 10th Joint Annual Meeting

18
**Sponsorship Application Form and Contract**

Please note that all acknowledgements of your company and listing of company name and address will be generated from the following information. It is mandatory to complete and sign this form to confirm your sponsorship. This form will be considered as a contract.

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#### Publication/Advertising (*exclusive)

- Advertising in the mini/pocket programme*
- Advertising in the Final Programme

#### Meeting Services (*exclusive)

- Speaker preview room* – CHF 5’000
- Internet corner*– CHF 5’000
- Smartphone application*– CHF 5’000

#### Promotional Material (*exclusive)

- Meeting bags sponsorship* – CHF 20’000
- Insert in Meeting bags – CHF 2’500 each
- Lanyards* – CHF 10’000
- Pens only – CHF 2’500
- Writing pads and pens – CHF 5’500

#### Social Event (*exclusive)

- Coffee Breaks* – CHF 5’000 each

### Payment Schedule

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Place and Date:  Signature and company stamp:
Cytokines 2012 –
10th Joint International Meeting of the International Cytokines Society and the International Society for Interferon and Cytokine Research

Personal Program
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# Overview

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Welcome address and Awards presentations  
*Tuesday, September 11, 2012 / 18:00 - 20:00*

**Room:** Room 2  
**Type:** Plenary session  
**Chairperson:** David Wallach (Israel), Charles Samuel (United States)

**Presentation:** Welcome address  
*Tuesday, September 11, 2012 / 18:00 - 18:15*

**Invited speaker:** Cem Gabay (Switzerland), Amanda Proudfoot (Switzerland)

**Presentation:** ICS and ISICR awards presentations  
*Tuesday, September 11, 2012 / 18:15 - 19:00*

**Presentation:** ICS Honorary life time membership award presentation  
Regulating the innate immune response with anti-TNF and ARAZ90  
*Tuesday, September 11, 2012 / 19:00 - 19:30*

**Invited speaker:** Anthony Cerami (Netherlands)

**Presentation:** ISICR Honorary life time membership award presentation  
Toward a genetic theory of infectious diseases  
*Tuesday, September 11, 2012 / 19:30 - 20:00*

**Invited speaker:** Jean-Laurent Casanova (United States)

---

**Abstract:**  
**CYTO12-1037: TOWARD A GENETIC THEORY OF INFECTIOUS DISEASES**  
J.-L. Casanova 1,*  
1THE ROCKEFELLER UNIVERSITY, New York, United States

The hypothesis that inborn errors of immunity underlie infectious diseases is gaining experimental support. However, the apparent modes of inheritance of predisposition or resistance differ considerably between diseases and between studies. A coherent genetic architecture of infectious diseases is lacking. We suggest here that life-threatening infectious diseases in childhood, occurring in the course of primary infection, result mostly from individually rare but collectively diverse single-gene variations of variable clinical penetrance, whereas the genetic component of predisposition to secondary or reactivation infections in adults is more complex. This model is consistent with (i) the high incidence of most infectious diseases in early childhood, followed by a steady decline, (ii) theoretical modeling of the impact of monogenic or polygenic predisposition on the incidence distribution of infectious diseases before reproductive age, (iii) available molecular evidence from both monogenic and complex genetics of infectious diseases in children and adults, (iv) current knowledge of immunity to primary and secondary or latent infections, (v) the state of the art in the clinical genetics of non-infectious pediatric and adult diseases, and (vi) evolutionary data for the genes underlying single-gene and complex disease risk. With the recent advent of new-generation deep resequencing, this model of single-gene variations underlying severe pediatric infectious diseases is experimentally testable.

**Disclosure of Interest:** None Declared
Keynote lecture

**Room:** Room 2  
**Type:** Plenary session  
**Chairperson:** Cem Gabay (Switzerland), Amanda Proudfoot (Switzerland)

**Presentation:** The roles of IL-17A and IL-17F in inflammation, tumorigenesis, and host defense against pathogens

Tuesday, September 11, 2012 / 20:00 - 20:30

Invited speaker: Yoichiro Iwakura (Japan)

MEET AND GREET

**Room:** Exhibition area  
**Type:** Breaks  
**Description:** All registered participants and registered accompanying persons are cordially invited to take the opportunity to meet with colleagues from all over the world, at the Meet and Greet reception.

Plenary session - Cytokines and infectious disease: host-pathogen interactions

Wednesday, September 12, 2012 / 08:30 - 10:30

**Room:** Room 2  
**Type:** Plenary session  
**Chairperson:** David Wallach (Israel), Yoichiro Iwakura (Japan)

**Presentation:** Type-2 innate lymphoid cells in protective immunity and asthma

Wednesday, September 12, 2012 / 08:30 - 09:00

Invited speaker: Andrew McKenzie (United Kingdom)

---

**Abstract:**

**CYTO12-1351: RORA AND NOTCH PLAY IMPORTANT ROLES IN TYPE-2 ILC DEVELOPMENT**

A. McKenzie 1*

1MRC-LMB, Cambridge, United Kingdom

**Abstract:** Dysregulation of type-2 immune responses, characterised by production of IL-4, IL-5, IL-9, and IL-13, underlies many aspects of allergic asthma. It is becoming increasingly clear that innate cell types, in addition to the classical Th2 cells, can produce these cytokines and that they may play critical roles in the initiation of the type-2 response. Using Il13-eGFP reporter mice, we identified and characterised a new innate type-2 immune effector leukocyte that we named the nuocyte. Nuocytes can be defined as lineage negative (lin\(^{neg}\)), ICOS\(^{+}\)IL-17BR\(^{+}\)ckit\(^{variable}\)T1/ST2\(^{variable}\) and represent the predominant early source of IL-13 during helminth infection with *Nippostrongylus brasiliensis*. Type-2 ILCs also expand in experimental models of asthma, where they may represent an important source of type-2 cytokines. We have now shown that nuocytes belong to the lymphoid lineage and can be included in the type-2 innate lymphoid cell (ILC) family. These type-2 ILCs develop from common lymphoid progenitors under signals from IL-7 and IL-33. Furthermore, we demonstrate that, like T cells, nuocytes require Notch signaling for development. We have gone on to show that the transcription factor RORα, a relative of RORγt, plays an important role in nuocyte development.

**Disclosure of Interest:** None Declared
Abstract:

CD28 is a homodimer expressed constitutively on T cells that functions as the principal costimulatory ligand in the immune response through an interaction with its B7 coligands. Hitherto, CD28 was not known to bind microbial components yet our finding is that bacterial superantigen toxins co-opt CD28 as their receptor and that to induce a cytokine storm comprised mainly of IL2, IFN-γ and TNF-α, the superantigen must bind, in addition to its classical ligands, MHC II and T cell receptor, directly into the homodimer interface of CD28. Preventing access of the superantigen to CD28 suffices to block its lethality. The interaction can be blocked with peptides that mimic the contact domains in superantigen or CD28. These peptides, we show, attenuate inflammatory cytokine gene induction and thus protect animals from lethal toxic shock. We elucidated the molecular mechanism of inflammatory cytokine storm underlying toxic shock, and used structural insight to design antagonists, one of which is now in a multi-center Phase II clinical trial against streptococcal toxic shock. We thus identify the CD28 homodimer interface as a critical receptor target for superantigens and key transducer of lethal cytokine storm. Our finding that CD28 is a receptor for the superantigen toxins broadens the scope of microbial pathogen recognition mechanisms and provides a novel approach for designing therapeutics that protect against the harmful effects of an inflammatory cytokine storm. Supported by DARPA, USAMRMC and a Biodefense Challenge Grant from NIAID.

Disclosure of Interest: None Declared
**Abstract:** The type I interferon (IFN) response protects cells from invading viral pathogens by triggering the transcription of interferon-stimulated genes (ISGs). Although hundreds of ISGs have been identified, relatively few have been characterized with respect to antiviral activity. For most, little is known about their antiviral potential, their target specificity, and their mechanisms of action. We have established an overexpression screening platform to evaluate the antiviral potential of more than 350 ISGs against a diverse panel of animal viruses spanning the viral phylogeny. To date, we have screened 13 plus-strand RNA viruses, 7 negative-strand RNA viruses, and one DNA virus. Hierarchical clustering of top ISG hits indicates that viruses segregate based on their genomic content, with plus-stranded RNA viruses exhibiting the highest sensitivity to the greatest number of ISGs. We have also probed the mechanisms of action of select antiviral ISGs against hepatitis C virus, dengue virus, and influenza A virus. Many of these ISGs feed back into antiviral programs, while others appear to have more targeted effector functions. Using a variety of experimental techniques, we show that inhibition of early viral life cycle events is a common mechanism targeted by numerous IFN effectors. Gaining additional insight into the cellular and biochemical mechanisms if ISG-mediated inhibition may provide a platform for the development of novel antiviral therapies.

**Disclosure of Interest:** None Declared

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**Presentation: C-type lectins, cytokines and host-fungal interactions**  
*Wednesday, September 12, 2012 / 10:00 - 10:30*

**Invited speaker:** Gordon Brown (United Kingdom)

**COFFEE BREAK**  
*Wednesday, September 12, 2012 / 10:30 - 11:00*

**Room:** Exhibition area  
**Type:** Breaks  
**Description:** *with the kind support of Roche*

**Disease-oriented symposium - Cytokine regulation of tissue remodeling**  
*Wednesday, September 12, 2012 / 11:00 - 12:30*

**Room:** Room 2  
**Type:** Disease oriented symposium  
**Chairperson:** Eleanor N. Fish (Canada)

**Presentation: Cytokine regulation of the bone**  
*Wednesday, September 12, 2012 / 11:00 - 11:30*

**Invited speaker:** Georg Schett (Germany)

**Presentation: Sclerostin - a key bone regulatory molecule**  
*Wednesday, September 12, 2012 / 11:30 - 12:00*

**Invited speaker:** Martyn Robinson (United Kingdom)

**Abstract:**  
**CYTO12-1336: SCLEROSTIN - A KEY BONE REGULATORY MOLECULE.**  
M. Robinson 1,
UCB Pharma, Slough, United Kingdom
Abstract: Sclerostin is an osteocyte-expressed, extracellular cystine-knot protein that is lacking in patients with sclerosteosis, a rare condition characterized by excessive bone formation. Sclerosteosis patients exhibit very high bone mass (lumbar spine Z scores up to +14) and are anecdotally resistant to bone fracture. Homozygous patients commonly develop symptoms associated with cranial nerve entrapment from excessive bone formation but do not show signs of heterotopic bone formation or metabolic abnormalities. Carriers demonstrate moderately elevated bone mass without associated untoward symptoms of excessive bone formation. Sclerostin knockout mice recapitulate many of the features of the excessive bone formation seen in sclerosteosis patients. Sclerostin acts by down modulating signaling through two osteogenic pathways (the Wnt and BMP pathways) and is consequently believed to negatively regulate the anabolic output from cells in the osteoblast lineage. Treatment of rodents with monoclonal antibodies that block the function of sclerostin results in significant increases in bone formation, bone mass and bone strength. Further, antibodies to sclerostin can reverse the bone loss in rodents associated with ovariectomy, chronic inflammatory conditions and steroids. In primates, sclerostin antibodies cause a dose-dependent increase in circulating markers of bone formation as well as increasing bone mineral density and bone strength in both trabecular and cortical bone. Sclerostin antibodies also accelerate bone repair in both rodent and primate fracture repair models. Mechanistic studies have shown that primarily acts to stimulate bone formation through a modeling rather than a remodeling pathway.

A Phase 1 clinical study demonstrated that a humanized antibody to sclerostin increased markers of bone formation and also inhibited a marker of bone resorption in a dose-dependent manner. Sclerostin inhibition provides an opportunity to evaluate therapies to increase bone mass to reduce fracture and accelerate fracture healing.

Disclosure of Interest: M. Robinson Shareholder of: UCB Pharma, Employee of: UCB Pharma

Presentation: REGULATION OF TISSUE REMODELLING BY ONCOSTATIN M IN MOUSE LUNG IS IL-6- AND SMAD3-INDEPENDENT

Wednesday, September 12, 2012 / 12:00 - 12:15

Abstract presenter: Carl Richards

Abstract:

CYTO12-1203: REGULATION OF TISSUE REMODELLING BY ONCOSTATIN M IN MOUSE LUNG IS IL-6- AND SMAD3-INDEPENDENT

F. Botelho 1, R. Rodrigues 2, J. Guerette 2, K. Ask 2, C. Richards 2,*
1Cornell University, Ithaca, New York, United States; 2MCMASTER UNIVERSITY, Hamilton, Canada

Introduction: Remodeling of extracellular matrix (ECM) in lungs occurs with various chronic inflammatory conditions including idiopathic pulmonary fibrosis and allergic airway disease. Although TGFbeta/SMAD signaling and other molecules are recognized as important in controlling ECM deposition, the gp130 cytokine (or IL-6/LIF cytokine) family including Oncostatin M (OSM) and IL-6 have also been implicated. OSM is a potent regulator of ECM in Balb/C and C57Bl/6 mouse lungs. Since OSM markedly induces IL-6 in vivo, here we tested the requirement for IL-6 using IL-6-/− mice and, in parallel, effects in SMAD3-/− mice in this system.

Methods: We used Adenovirus vector encoding murine OSM (AdOSM), to over-express OSM (endothelaechal administration) and examined accumulation of collagen in lung tissue by histology, mRNA levels, chemokine levels in lung lavage and recruitment of CD45+collagen1+ fibrocytes using Flow cytometry of whole lung mononuclear cell populations, with an n=5 for each treatment in the separate mouse lines.

Results: In wild type mice, using empty vector Addel70 as a control treatment, AdOSM markedly induced mRNA levels of collagen1A1, 1A2, elastin, fibronectin, MMP13, TIMP−1, and IL-6 at Days 5 and 7 while TIMP-3 was reduced. Activated STAT3 was evident at day 2 and 7 as assessed by Western blots of individual mouse lung homogenates and immuno-histochemistry of tissue sections.
Presentation: A NOVEL FUNCTION OF INTERFERON BETA IN PROMOTING THE GENERATION OF ANTI-INFLAMMATORY M2-LIKE MICROGlia IN VITRO AND IN VIVO

Wednesday, September 12, 2012 / 12:15 - 12:30

Abstract presenter: Jui-Hung Yen

Abstract:

CYTO12-1077: A NOVEL FUNCTION OF INTERFERON BETA IN PROMOTING THE GENERATION OF ANTI-INFLAMMATORY M2-LIKE MICROGLIA IN VITRO AND IN VIVO

J.-H. Yen 1,*, D. Ganea 1
TEMPLE UNIVERSITY SCHOOL OF MEDICINE, Philadelphia, United States

Introduction: IFNβ is an approved therapeutic option for the treatment of the autoimmune multiple sclerosis (MS). The molecular mechanisms underlying the effects of IFNβ in MS ant its animal model experimental autoimmune encephalomyelitis (EAE) are not fully understood and especially the effects of IFNβ in the CNS cells are largely unknown. In this study, we identified a novel mechanism for the beneficial effect of IFNβ in MS/EAE through the conversion of microglia (MG) from pro-inflammatory M1-like MG to anti-inflammatory M2-like MG.

Methods: For in vitro study: Neonatal MG were generated and activated with LPS (1μg/ml) in the presence or absence of IFNβ (1,000U/ml) for 3h and 6h. Cells were then harvested and subjected to RNA extraction, followed by Q-PCR for IL-1β, IL-12p40, IL-23p19, TNFα and IL-10. To measure secreted cytokine production, the supernatants from cells treated with LPS in the presence or absence of IFNβ for 24h were collected and subjected to ELISA. To determine the effect of IFNβ-treated MG on Th1 and Th17 activation, MG matured with LPS in the presence or absence of IFNβ were co-cultured with T cells purified from spleens of 2D2 mice for 3 days. The production of IFNγ and IL-17 was measured by both intracellular staining and ELISA.

For in vivo study: Mice (n=12) were immunized with MOG35-55 and administrated with either IFNβ (10,000U/ml, n=6) or vehicle (n=6) every other day starting from day 5 after immunization. Clinical scores were recorded at day 15. Spinal cords and brains from EAE mice were subjected to Iba-1 immunostaining or RNA extraction, followed by Q-PCR for Arginase1, YM-1 and IL-10 expression.

Results: Our results show that the expression and production of proinflammatory M1-type cytokines, such as IL-1β, IL-12p40, IL-23 and TNFα were inhibited by IFNβ and the production of anti-inflammatory M2 cytokine IL-10 was enhanced by IFNβ in LPS-stimulated primary MG. Through this phenotype switch, IFNβ-treated MG suppressed Th1 and Th17 activation leading to the downregulation of IFNγ and IL-17 production. In vivo, IFNβ-treated EAE mice exhibit lower clinical scores, reduced Iba-1 expression, and increased expression of the M2 markers, arginase, YM-1 and IL-10 in the CNS.

Conclusion: Taken altogether, our results demonstrate for the first time that IFNβ favors the generation of MG2 leading to the secretion of the anti-inflammatory cytokine IL-10 and reduced expression of the pro-inflammatory cytokines, IL-12, IL-23, IFNγ and IL-17 that provides a novel molecular mechanism for the beneficial effect of IFNβ in MS/EAE.
Disclosure of Interest: None Declared

**Disease-oriented symposium - IFN treatment of viral infections**  
*Wednesday, September 12, 2012 / 11:00 - 12:30*

**Room:** Room 3&4  
**Type:** Disease oriented symposium  
**Chairperson:** Leonidas Platanias (United States)

**Presentation:** Host-virus interactions in acute and chronic hepatitis C  
*Wednesday, September 12, 2012 / 11:00 - 11:30*

**Invited speaker:** Marcus Heim (Switzerland)

**Abstract:** CYTO12-1307: HOST-VIRUS INTERACTIONS IN ACUTE AND CHRONIC HEPATITIS C  
M. Heim 1,*  
UNIVERSITY BASEL, Basel, Switzerland

**Abstract:** Interferons are not only the first line of defense against viral infections such as hepatitis C virus infections, but also have important roles during the chronic phase of viral infections. For over 20 years now, recombinant interferon alpha has been used for the treatment of chronic hepatitis C. The molecular mechanisms responsible for non-response to interferon are still not completely understood, but the systematic analysis of liver biopsies revealed that the spontaneous activation of the endogenous interferon system in the liver of patients with chronic hepatitis C prevented response to interferon-based therapies. Moreover, recent genome wide association studies found a highly significant and strong association between genetic variants near the IFNλ3 gene, designated the IL28B genotype, with spontaneous clearance of hepatitis C virus as well as with response to treatment of chronic hepatitis C with pegylated interferon alpha and ribavirin. The molecular pathways that link the IL28B genotype with antiviral effector systems of the innate and adaptive immune system are not known. However, substantial progress has been made in the basic understanding of the induction of interferons through toll-like receptor and RIG-I/MDA5 pathways, and of interferon induced signaling pathways and antiviral effector systems. Over the last two decades, hepatitis C virus has been an important tool to study fundamental aspects of host-virus interactions in a chronic viral infection. Further insights into the viral escape strategies that allow hepatitis C virus to persist for decades despite an ongoing innate and adaptive immune response will eventually allow the rational development of preventive vaccines.

**Disclosure of Interest:** None Declared

**Presentation:** Clinical experience with interferon-alpha in hepatitis C: past, present and future  
*Wednesday, September 12, 2012 / 11:30 - 12:00*

**Invited speaker:** Francesco Negro (Switzerland)

**Presentation:** INTERFERON ALPHA SUBTYPE 11 ACTIVATES NK CELLS AND ENABLES CONTROL OF RETROVIRAL INFECTION  
*Wednesday, September 12, 2012 / 12:00 - 12:15*

**Abstract presenter:** Kathrin Gibbert
Abstract:
CYTO12-1135: INTERFERON ALPHA SUBTYPE 11 ACTIVATES NK CELLS AND ENABLES CONTROL OF RETROVIRAL INFECTION
K. Gibbert 1*, J. J. Joedicke 1, A. Meryk 1, A. Kraft 1, K. S. Lang 1, U. Dittmer 1
1UNIVERSITY DUISBURG-ESSEN, Essen, Germany

Introduction: Type I Interferons (IFNs) are a multigene family with up to 14 different IFNα subtypes. IFNs have direct anti-viral activity mediated by the induction of anti-viral enzymes, but they also stimulate cells of the innate and adaptive immune system. Various studies demonstrated distinct anti-viral activities of specific IFNα subtypes but their immunomodulatory properties during viral infections have not been investigated. The innate immune response mediated by cells such as natural killer (NK) cells is critical for the rapid containment of virus replication and spread during acute infection. In this study we are interested on the specific immunomodulatory effects of various IFNα subtypes on NK cell functions during retroviral infections.

Methods: We used different IFNα subtypes for immunotherapy of acute Friend retrovirus (FV) infection in mice to study their immunomodulatory effects in vivo. For that purpose, we did FACS analysis of different lymphocyte populations, analysis of viral loads, cell depletion experiments, adoptive transfer and in vitro cytotoxicity assays.

Results: Treatment of mice with IFNα11 during FV infection significantly reduced viral loads and resulted in long-term protection from virus-induced leukemia. The effect of IFNα11 on NK cells was direct and signaled through the type I IFN receptor. Furthermore, IFNα11-mediated activation of NK cells enabled cytolytic killing of FV-infected target cells via the exocytosis pathway. Depletion and adoptive transfer experiments illustrated that NK cells played a major role in successful IFNα11 therapy. Additional experiments with Mouse Cytomegalovirus infections demonstrated that the therapeutic effect of IFNα11 is not restricted to retroviruses. The type I IFN subtypes 2 and 5, which bind the same receptor as IFNα11, did not elicit similar antiviral effects.

Conclusion: These results demonstrate a unique and subtype-specific activation of NK cells by IFNα11.

Disclosure of Interest: None Declared

Presentation: USING TYPE III INTERFERON TO IMPROVE THE EFFICACY OF VACCINE AND ONCOLYTIC VIRAL VECTORS

Wednesday, September 12, 2012 / 12:15 - 12:30

Abstract presenter: Ryann C. Guayasamin

Abstract:
CYTO12-1149: USING TYPE III INTERFERON TO IMPROVE THE EFFICACY OF VACCINE AND ONCOLYTIC VIRAL VECTORS
T. D. Reynolds 1, R. C. Guayasamin 1, M. Fujiwara 1, M. D. Robek 1
Yale School of Medicine, New Haven, United States

Introduction: Vaccines based on replicating viral vectors that express foreign antigens can generate superior durable antibody and memory CD8 T cell responses compared to inactivated virus or recombinant protein, with the added benefit of potential needle-free intranasal administration. Despite these significant advantages, safety concerns related to viral vectors as vaccine platforms can be an issue with this approach. Replicating viral vectors are perceived as less safe alternatives compared to other immunization modalities, especially in the context of intranasal administration, where the potential exists for systemic virus spread from the lung. The Type III interferons (IL-29, IL-28A, IL-28B; also known as IFN-lambda1, 2, and 3) play a critical role in protecting epithelial cells in the lung and gut from virus infection, and can also stimulate adaptive immunity. We therefore examined the ability of this cytokine family to improve the safety and efficacy of a viral vector by limiting its systemic spread and vector-associated pathological effects after intranasal delivery.
Methods: Vesicular stomatitis virus (VSV) based vectors are promising candidates for use as vaccines and oncolytic agents. We generated recombinant VSV vectors expressing mouse IL-28A either from the fifth position or the more highly expressing first position of the viral genome, and measured virus replication in cell culture and mice.

Results: We found that IL-28A is properly expressed, secreted, and active when produced by VSV-infected cells. In cell culture, expression of IL-28A from VSV attenuated virus replication in type III IFN-sensitive cells, but not in type III IFN-resistant cells. Following intranasal inoculation in mice, spread of the VSV.IL28A vector from the lung to the lymph nodes, spleen, blood, liver, and brain was significantly reduced compared to recombinant wild-type VSV. Despite this attenuation, the VSV.mIL28A vector generated virus-specific CD8 T cell and antibody responses in mice that were comparable to wild-type VSV.

Conclusion: These studies demonstrate the ability of the type III interferon cytokine family to improve the safety and efficacy of a clinically promising viral vaccine vector. These results may potentially also inform the enhancement of VSV oncolytic activity by increasing selectivity of the virus for type III IFN-resistant tumor cells.

Disclosure of Interest: None Declared

Parallel session - Cytokines and innate immune responses

Wednesday, September 12, 2012 / 14:00 - 16:00

Room: Room 2
Type: Parallel session
Chairperson: Nancy Reich (United States)

Presentation: Processing of IL-1 family cytokines by cell death-related proteases

Wednesday, September 12, 2012 / 14:00 - 14:30

Invited speaker: Seamus Martin (Ireland)

Abstract:

CYTO12-1338: AN ALTERNATIVE ROUTE TO IL-1B PROCESSING GENERATES AN IL-1 RECEPTOR ANTAGONIST

S. Martin 1,*
TRINITY COLLEGE DUBLIN, Dublin, Ireland

Abstract: IL-1 plays a central role in the initiation of immune responses and is expressed as an inactive precursor protein, requiring limited internal proteolysis to produce the bioactive cytokine. While caspase-1 has been established as a major IL-1b activating enzyme, the cytotoxic lymphocyte protease granzyme A has also been reported to process and activate IL-1b. However, we have found that granzyme A-dependent processing of IL-1b not only failed to activate this cytokine but, surprisingly, converted this protein into an IL-1 receptor antagonist. Our data reveal an exquisitely subtle mechanism for blunting IL-1-dependent responses and suggest that, in addition to the central role of caspase-1 in promoting IL-1b activation, proteolysis may also serve as a regulatory step in the inactivation of this cytokine.

Disclosure of Interest: None Declared

Presentation: IL-36: Immune effects and role in psoriasis

Wednesday, September 12, 2012 / 14:30 - 15:00

Invited speaker: John Sims (Switzerland)

Abstract:
CYTO12-1357: IL-36 STIMULATES TH1 RESPONSES AND IS IMPLICATED IN HUMAN PSORIASIS
J. Sims 1*
UNIVERSITY OF GENEVA, Geneve, Switzerland

Abstract: IL-36, IL-36, IL-36 and their antagonist IL-36Ra are members of the IL-1 family which are expressed in skin, airway, upper GI tract and other epithelial tissues. Elevated expression of IL-36 has been strongly linked to human plaque psoriasis based on the following evidence: (a) IL-36 and IL-36 are dramatically upregulated in the lesional skin of psoriasis patients; (b) transgenic over-expression of IL-36 in mouse skin results in an inflammatory condition strongly resembling psoriasis; and (c) human lesional skin transplanted onto an immunodeficient mouse is largely normalized by an anti-human IL-36R antibody. Interestingly, humans suffering from a psoriasis variant, generalized pustular psoriasis, have been shown to carry homozygous loss-of-function mutations in IL-36Ra. Because T cells are important in the pathology of psoriasis, we have begun to explore the effects of IL-36 on T cell function. IL-36R is highly expressed in naïve Th0 cells, but not in Th1, Th2 or Th17 helper T cell subsets. Stimulation of activated Th0 cells with IL-36 leads to proliferation and IL-2 production but not to induction of polarized T cell cytokines. However, incubation of activated Th0 cells and bone marrow-derived dendritic cells with IL-36 results in substantial IFN production. The critical role of dendritic cells is to provide IL-12 to the T cells, and the combination of IL-12 and IL-36 leads to three times stronger production of IFN than does the combination of IL-12 and IL-18. This response is also relevant in vivo, since IL-36R/- mice infected with Mycobacterium Calmette-Guerin generate weaker IFN responses and show more pathology than similarly-infected wild-type mice.

Disclosure of Interest: J. Sims Shareholder of: Amgen, Employee of: Amgen (past)

Presentation: PLATELET EXPRESSION OF MIF AND ITS SECRETION BY THROMBOGENIC STIMULATION
Wednesday, September 12, 2012 / 15:00 - 15:15

Abstract presenter: Sabine Tillmann

Abstract: CYTO12-1227: PLATELET EXPRESSION OF MIF AND ITS SECRETION BY THROMBOGENIC STIMULATION
S. Tillmann 1*, T. Strüßmann 1*, T. Wirtz 1*, J. Bernhagen 1
1Institute of Biochemistry and Molecular Cell Biology, Aachen, Germany

Introduction: Atherosclerosis, a chronic inflammatory disease of the arterial wall, is the main underlying pathology of cardiovascular diseases in the Western world. Chemokines in conjunction with leucocytes recruited into the atherogenic vessel wall are key players in this process. It also turned out that platelets have an important impact on the course of the disease, for example through the secretion of a variety of growth factors and inflammatory chemokines such as platelet factor 4 (PF4/CXCL4), RANTES/CCL5, or SDF-1α/CXCL12 [1]. Despite this knowledge, the precise contributions of the involved chemokines are incompletely understood and it has been assumed that platelets contain additional inflammatory factors. We recently demonstrated that the chemokine-like function chemokine macrophage migration inhibitory factor (MIF) has a key role in atherogenic lesion development. MIF is secreted from atherogenic endothelium and activated macrophages and promotes monocyte and T cell recruitment through non-cognate interaction with CXC chemokine receptors [2]. Here we asked whether platelets express MIF and whether platelet-derived MIF would contribute to atherogenic plaque development.

Methods: Detection and quantification of postulated MIF stores in platelets was performed by Western Blot analysis and ELISA. The presence of MIF in K652 cells differentiated into megakaryocytes was studied by confocal microscopy. MIF secretion was measured after activation of purified human platelets with different stimuli and MIF content in platelet supernatants was quantified by human MIF
ELISA. qPCR was used to determine mRNA contents in the megakaryocytic cell line and in primary human platelets.

**Results:** We were able to demonstrate the presence of appreciable amounts of MIF protein in both human and murine platelets. Furthermore, the K562 megakaryocyte line stained positive for MIF. We quantified the amount of MIF in human platelets to be around 0.3 fg MIF/platelet. Moreover, qPCR demonstrated the presence of MIF mRNA in differentiated K562 cells and, although at a low copy number, even in primary human platelets. To begin to examine whether platelet MIF might contribute to local inflammatory processes such as in the atherogenic vessel, MIF secretion from purified human platelets in response to various stimuli was measured by ELISA. After thrombin stimulation, 70% of the endogenous MIF was secreted compared to unstimulated platelets as early as 4 h after stimulation. Likewise, collagen stimulation led to a significant MIF release (paired Student’s t-test; significance: p<0.05). In contrast to these well-known thrombogenic stimuli, potential atherogenic stimuli like oxLDL or TNF-a did not lead to measurable MIF levels in the platelet supernatants.

**Conclusion:** This is the first report demonstrating MIF secretion from platelets upon thrombogenic activation, suggesting that platelets could be a previously unrecognized source of MIF in the local atherogenic environment of an inflamed vessel.

**References:**

**Disclosure of Interest:** None Declared

**Presentation:** ROLE OF NUCLEIC ACID-BINDING POLYMERS IN TLR ACTIVATION AND AUTOIMMUNE DISEASE DEVELOPMENT

**Abstract presenter:** Eda Holl

**Abstract:**
CYTO12-1047: ROLE OF NUCLEIC ACID-BINDING POLYMERS IN TLR ACTIVATION AND AUTOIMMUNE DISEASE DEVELOPMENT
E. Holl 1, B. Sullenger 1
1DUKE UNIVERSITY, Durham, United States

**Introduction:** Toll-like receptors (TLRs) play a critical role in innate and adaptive immune responses by responding to pathogenic nucleic acids. Moreover, they have recently been shown to play a role in the pathogenesis of autoimmune disorders such as systemic lupus erythematosus (SLE) due to their ability to recognize self-antigens. There has been growing evidence suggesting that blocking of nucleic acid-sending TLRs such as TLR7 and 9 results in ameliorated disease. Although effective in blocking autoimmune disease progression, TLR inhibitors are not ideal given that they interfere with normal responses to pathogens. Our goal is to create compounds that bind nucleic acids prior to their entry into the cell and engagement of TLRs. Here we determined the role of nucleic acid-binding cationic polymers in neutralizing the proinflammatory effects of nucleic acids on a variety of immune cells implicated in autoimmune disease development.

**Methods:** B cells and dendritic cells (DCs) of wild type and lupus prone mice were exposed to TLR7 and 9 synthetic agonists. Supernatants were subsequently assessed for nucleic acid-driven proinflammatory cytokine release, plasma cell differentiation and antibody production. Cells were also assessed for their ability to respond to encapsulated viral particles in the presence of nucleic-acid binding cationic polymers.
Results: Nucleic acid-binding polymers inhibit TLR activation and subsequent cytokine production by both dendritic cells (DCs) and B cells of wild type and lupus prone mice. They also block nucleic acid-driven plasma cell differentiation and antibody production. Additionally, they modulate co-stimulatory cell surface marker expression (CD80, CD86) in stimulated DCs and B cells. Stimulation of immune cells by encapsulated viral particles is unaffected in the presence of polymers.

Conclusion: These findings provide a new avenue in drug development as nucleic acid-binding agents can potentially be utilized to block overt autoimmune disorders while allowing normal immune responses to occur.

Disclosure of Interest: None Declared

Presentation: IRF-5; MEDIATES CROSS TALK BETWEEN INNATE AND ADAPTIVE IMMUNITY

Wednesday, September 12, 2012 / 15:30 - 15:45

Abstract presenter: Paula Pitha Rowe

Abstract:

CYTO12-1298: IRF-5; MEDIATES CROSS TALK BETWEEN INNATE AND ADAPTIVE IMMUNITY

P. Pitha Rowe 1,*, S. Roy 1

Johns Hopkins University, Baltimore, United States

Introduction: IRF-5 is a transcription factors activated by TLR7 and TLR9 during innate immune responses. IRF-5 activates not only Type I IFN, but also inflammatory cytokines and chemokines. Most importantly a distinct genetic variations in the IRF-5 gene show a strong association with autoimmune diseases such as Lupus (SLE). Here we analyzed the the role of IRF-5 in the induction of inflammatory cytokines and B cells activation, two hallmarks of SLE.

Methods: The two strains of IRF-5 knockout mice were evaluated for their response to antigens in vivo. The IgG switching was anlayzed in isolated B cells in vitro. The molecular mechanism involved in the alteration of the antigenic response was determined on the molecular levels.

Results: Using two independently generated strains of IRF5-deficient mice Irf5-/- mice we showed that these mice exhibit high susceptibility to VSV and HSV-1 infection, reduction in serum levels of type I IFN as well as inflammatory cytokines, including interferon in response to viral infection, high susceptibility to virus (VSV) and parasite (Laishmania donovai) infection and a defect in generation of the TH-1 response. IRF-5, but not IRF-7, is also critical for B cells differentiation to plasma cells, IgG2a/c responses to antigens and to polyoma virus infection. This defect is due to the intrinsic deletion of IRF-5 in B cells, as Rag1 knockout mice reconstituted with Irf5-/- B cells show a decrease in IgG2a/c expression after viral infection. Irf5-/- B cells in vitro have diminished TLR and cytokine-induced class switching to IgG2a/c. Addressing the molecular mechanism of these defects, we show that IRF-5 regulates Blimp-1 and Ikaros expression, factors essential for plasma cells differentiation and IgG2a/c expression respectively. Reconstitution of IRF-5 in Irf5-/- B cells rescue Blimp-1 expression, down-regulates Ikaros levels and increases switching to IgG2a/c. The IRF site in Blimp-1 and ikzf1 promoters binds IRF-5.

Conclusion: Collectively these results identify the role of IRF-5 both in the innate and adaptive immune response and indicate its a critical role in the cross talk between the innate and adaptive immune responses.

Disclosure of Interest: None Declared

Presentation: TYPE I INTERFERONS LICENSE CASPASE-11-DEPENDENT NLRP3 INFLAMMASOME ACTIVATION BY GRAM-NEGATIVE BACTERIA

Wednesday, September 12, 2012 / 15:45 - 16:00
Abstract presenter: Vijay Rathinam

Abstract: CYTO12-1163: TYPE I INTERFERONS LICENSE CASPASE-11-DEPENDENT NLRP3 INFAMMASOME ACTIVATION BY GRAM-NEGATIVE BACTERIA
V. Rathinam 1, S. Kailasan Vanaja 2, L. Waggoner 1, A. Sokolovska 3, C. Becker 3, L. Stuart 3, J. Leong 2, K. Fitzgerald 1
University of Massachusetts Medical School, Worcester, 2Tufts University School of Medicine, 3Mass General Hospital, Boston, United States

Introduction: Type I interferon response represents the hallmark of innate immune effector mechanisms. Although it has been well-documented that type I interferons are key players in coordinating anti-viral immunity, their role in bacterial infections remains poorly defined. Systemic infections with bacteria are characterized by high mortality rates due to the “sepsis syndrome”, a widespread and uncontrolled inflammatory response. The objective of this study was to determine the role of type I interferons in eliciting innate immune defense against bacterial pathogens. Here, we have identified a novel type I interferon pathway that licenses NLRP3 inflammasome activation by all Gram-negative bacteria.

Methods: Various knock-out mice and macrophages from them were used. Immunoblotting, ELISAs and qPCR were used to assess immune responses.

Results: A systematic investigation of inflammasome activation by enterohemorrhagic Escherichia coli (EHEC) and Citrobacter rodentium, Gram-negative enteropathogens, revealed a role for TLR4 and TRIF in NLRP3 inflammasome activation as macrophages lacking TLR4 or TRIF failed to process caspase-1 and secrete IL-1β and IL-18. TRIF signaling downstream of TLR4 triggers interferon regulatory factor (IRF)-3 dependent IFN-β production. Indeed, TLR4-TRIF signaling was found to be essential for IFN-β production elicited by EHEC and C. rodentium. Importantly, we found that EHEC and C. rodentium-induced IFN-β subsequently targets the activation of caspase-11, which is a member of the caspase-1 subfamily of inflammatory proteases. A recent study identified caspase-11 as a key regulator of caspase-1 activation and IL-1β/IL-18 production in response to a set of enteric pathogens (Kayagaki et al., 2011). Unlike other caspases, caspase-11 is unique in that it is highly inducible. We determined that type I interferon regulates caspase-11 activation by up-regulating caspase-11 expression in an autocrine/paracrine manner in EHEC and C. rodentium infection. Remarkably the transcriptional induction of caspase-11 by IFN-β was both necessary and sufficient to promote caspase-11 auto-activation suggesting a model wherein transcriptional induction of pro-caspase-11 is coupled to its auto-activation. Caspase-11 activation via the TLR4-TRIF-IFN-β pathway ultimately synergizes with the NLRP3 pathway to coordinate caspase-1 dependent IL-1β and IL-18 processing and secretion. The requirement for IFN-β-caspase-11 axis for NLRP3 inflammasome activation was found to be highly specific for Gram-negative but not Gram-positive bacteria. Finally, in a well-established acute bacterial peritonitis and shock model using E. coli, we determined that IFN-β-caspase-11 pathway is essential for E. coli induced IL-1β and IL-18 production not only in vitro but also in vivo.

Conclusion: Thus, the identification of type I interferon as a regulator of caspase-11-dependent NLRP3 inflammasome activation provides new insights into the integration of TLR and NLR pathways during Gram-negative bacterial infections and unveils new targets that might be manipulated to prevent uncontrolled inflammation during septic shock.


Disclosure of Interest: None Declared

Poster session I
with the kind support of Abbott

Room: Poster area
Type: Poster session

Wednesday, September 12, 2012 / 16:00 - 18:00
**Description:** Have an informal face-to-face meeting with researchers, students and colleagues who will be the utmost pleased to share their work and latest innovations with you, through poster presentation. Light snacks and refreshments will be provided.

Have a look in the "Poster" menu on the left for a list of the presented posters.

**Plenary session - Interferons and innate immunity**

*Thursday, September 13, 2012 / 08:00 - 10:00*

**Room:** Room 2

**Type:** Plenary session

**Chairperson:** Ganes Sen (United States), Marcus Heim (Switzerland)

**Presentation: Role of type I IFNs in priming and mediating PRR mediated innate immune responses**

*Thursday, September 13, 2012 / 08:00 - 08:30*

**Invited speaker:** Paul Hertzog (Australia)

**Presentation: RIG-I signaling in RNA virus infection and immunity**

*Thursday, September 13, 2012 / 08:30 - 09:00*

**Chairperson:** Stacy M. Horner

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**Abstract:**

**CYTO12-1333: RIG-I SIGNALING IN RNA VIRUS INFECTION AND IMMUNITY**

S. M. Horner

University of Washington, Seattle, United States

**Abstract:** RIG-I-like receptors (RLRs) are a class of cytosolic pathogen recognition receptors including RIG-I, MDA5, and LGP2. RIG-I and MDA5 are essential for triggering immunity towards distinct RNA viruses, while LGP2 may function as a cofactor or regulator of these processes. Our studies have shown that RIG-I in particular initiates immune defenses against hepatitis C virus (HCV), a major human pathogen. RIG-I engages pathogen-associated molecular pattern motif(s) within HCV RNA in infected cells to trigger innate immune defenses through its adaptor protein MAVS/IPS-1. However, HCV blocks RIG-I signaling through the viral NS3/4A protease cleavage of MAVS to support persistent infection. While MAVS resides on mitochondria and peroxisomes, how its signaling is coordinated among these organelles has been unknown. We have found that the mitochondrial-associated ER membrane (MAM), a distinct membrane subdomain which links ER to mitochondria, is a major site of MAVS localization and intracellular signaling of innate immune defenses against RNA viruses, including HCV. Upon RIG-I pathway activation, RIG-I is recruited to the MAM to bind MAVS. The MAM then dynamically organizes mitochondria and peroxisomes into MAVS-anchored synapses to regulate antiviral signaling. The importance of the MAM in anchoring these "innate immune synapses" is highlighted by the fact that the HCV NS3/4A protease cleaves MAVS on the MAM, but not the mitochondria, to evade immunity. Thus, an intracellular immune synapse anchored by the MAM coordinates innate immunity during RNA virus infection and is targeted by the HCV NS3/4A protease. While the interaction between RIG-I and MAVS at the MAM is essential for regulated innate immune signaling, it is unclear how RIG-I, a cytosolic protein, can identify and interact with MAVS-localized MAVS. We have also focused on defining the mechanisms of RIG-I translocation to the MAM for intracellular signaling. We now show that RIG-I interacts with the E3 ubiquitin ligase TRIM25 and the molecular chaperone 14-3-3ε to form a translocon that moves RIG-I from cytosol to MAM during the acute phase of RNA virus infection. Our results demonstrate that RIG-I signaling is mediated through this ternary complex of RIG-I/TRIM25/14-3-3ε to mediate engagement of RIG-I with MAVS to initiate signaling of immunity to HCV infection. Overall, our studies indicate that the RIG-I translocon-MAM signaling network is essential for immunity against HCV and other pathogenic RNA viruses.

**Disclosure of Interest:** None Declared
**Presentation: New insights on regulation of RIG-I signaling**
*Thursday, September 13, 2012 / 09:00 - 09:30*

**Invited speaker:** Adolfo Garcia-Sastre (United States)

**Presentation: Interferons: overlooked regulators of innate and adaptive immune responses**
*Thursday, September 13, 2012 / 09:30 - 10:00*

**Invited speaker:** Eleanor N. Fish (Canada)

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**Abstract:**

**CYTO12-1332: INTERFERONS: OVERLOOKED REGULATORS OF INNATE AND ADAPTIVE IMMUNE RESPONSES**

E. N. Fish ¹, ²,*
University Health Network, University of Toronto, Toronto, Canada

**Abstract:** Type I IFNs, that include the IFN-αs and IFN-β, are critical effectors of the host antiviral immune response. Much attention has focused on their direct antiviral effects against viruses in infected tissues, examining specific signaling cascades and effector molecules induced by these IFNs that mediate virus inhibition and protection from infection. To date, their activities as modulators of innate and adaptive immune responses have been largely overlooked, perhaps contributing to their limited clinical application as therapeutics for acute viral infections.

Our early studies using IFN-β⁻/⁻ mice revealed diverse consequences of IFN-β for both the innate and adaptive arms of immunity: altered splenic architecture in IFN-β⁻/⁻ mice and a reduction in resident macrophages; a defect in B cell maturation; circulating IgM-, Mac-1-, and Gr-1-positive cells are also substantially decreased in IFN-β⁻/⁻ mice. The decrease in the numbers of circulating macrophages and granulocytes likely reflects defective maturation of primitive BM hematopoiesis in mice, shown by a reduction in CFU-GM. Viewed together, these data indicate that IFN-β is required during different stages of maturation in the development of the immune system.

Constant immunosurveillance in the host requires that lymphocytes traffic through lymph nodes (LNs) to sample antigen. pDCs and lymphocytes use similar mechanisms for retention within LNs and we provided evidence that these processes are influenced by IFN-β, even in the absence of viral infection, implicating IFN-β in affecting critical APC-T cell interactions that determine a robust immune response to pathogen infection.

Most recently, we have conducted studies in mice infected intranasally with different strains of influenza A virus and show that clearance of virus mediated by IFN-β is associated with polarization of CD4 and CD8 T cells to a Th1 phenotype, increased DC infiltrates into the lungs and dLNs of infected mice, reduced eosinophilia, and a reduction in a novel antigen presenting cell population associated with type 2 immunity and lung immunopathology.

Viewed in conjunction with data that suggest an adjuvant role for IFNs in vaccination, accumulating evidence suggests that the immunomodulatory activities of type I IFNs contribute significantly to their antiviral effectiveness.

**Disclosure of Interest:** None Declared

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**Disease-oriented symposium - IL-17 & related cytokines in inflammatory diseases**
*Thursday, September 13, 2012 / 10:30 - 12:00*

**Room:** Room 2

**Type:** Disease oriented symposium

**Description:** with the kind support of Amgen
Chairperson: Federica Sallusto (Switzerland)

Presentation: IL-17 and related cytokines in arthritis  
*Thursday, September 13, 2012 / 10:30 - 11:00*

Invited speaker: Wim van den Berg (Netherlands)

Presentation: Th17 cells versus non-canonical IL-17 expressing innate immune cells in human inflammatory diseases  
*Thursday, September 13, 2012 / 11:00 - 11:30*

Invited speaker: Dominique Baeten (Netherlands)

Presentation: METASTATIC LEISHMANIASIS—AN IL-17 MEDIATED RESPONSE TO LEISHMANIA-VIRUS.  
*Thursday, September 13, 2012 / 11:30 - 11:45*

Abstract presenter: Mary-Anne Hartley

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**Abstract:**

**CYTO12-1319: METASTATIC LEISHMANIASIS—AN IL-17 MEDIATED RESPONSE TO LEISHMANIA-VIRUS.**  
M.-A. Hartley ³, C. Ronet ¹, N. Fasel ¹  
University of Lausanne, Epalinges, Switzerland

**Introduction:** As veterans of infection, *Leishmania guyanensis* parasites have been plaguing humankind for centuries, provoking a deleterious hyper-inflammatory response, destroying host tissue and forming the ulcerating lesions, which typify most forms of the disease. About 15% of patients develop secondary lesions in the mouth and nose, where parasites metastasise to mucocutaneous tissues creating corrosive and exceptionally disfiguring inflammation. Our lab has recently linked disease severity in these infections to a virus naturally residing within the cytoplasm of some leishmania parasites. Here, *Leishmania* RNA virus (LRV) can act as an independently immunogenic entity, where its RNA-based nucleic acid acts as a potent innate immunogen, triggering a destructive hyper-inflammatory cascade through Toll-Like-Receptor 3 recognition (1). The immune effectors underlying the devolution of stubborn and metastatic leishmaniasis of have largely focused on the Th1/Th2 (healing/non-healing) dogma: a paradigm, which neglects the role and interactions of other T-cell subsets, such as Th17.

**Methods:** Using *Leishmania guyanensis* clones, which are either naturally infected by LRV (V⁺) or depleted in it (V⁻), we set out to characterise the perpetrators of this chronic hyper-inflammation in a murine model of infection. These candidates were then used as targets for immunomodulatory intervention, with the aim of reaching a therapeutic end.

**Results:** Here, we found that V⁺ parasites potently induced the production of IL-17A as compared to their V⁻ equivalents, thus insinuating that this pro-inflammatory cytokine plays a destructive role in the devolution and severity of metastatic leishmaniasis. Indeed, IL-17A⁺ mice infected with V⁺parasites have a significantly reduced disease severity and parasite burden compared to wild type controls. Further, *in vitro* studies revealed that IL-17A directly increases the parasitism of macrophages while simultaneously decreasing their ability to clear the infection.

SR-1001: a recently described drug inhibiting the differentiation of Th17 cells, has been used in a pioneering study that inhibited the development of destructive inflammation in multiple sclerosis, we propose this immunomodulator (and its cheaper equivalent Digoxin), for trials in the treatment of complicated metastatic leishmaniasis.
Conclusion: Although IL-17 has a promiscuous role in immunity, stimulating both the innate and adaptive immune systems (as well as a plethora of non-immune cells), a major shared goal of these pathways is their self-propagation, where down-stream effects converge to create a hyper-inflammatory feedback loop. Indeed, the Th17 T-cell population has been thoroughly vilified as the architect of many chronic and destructive inflammatory processes. Determining the role of IL-17 in LRV-based virulence is essential to our understanding of its pathogenesis and would stand to guide and justify a much-needed immunotherapeutic revolution in the treatment of complicated leishmaniasis.

Disclosure of Interest: None Declared

Presentation: CRITICAL ROLE OF TH17 PRO-INFLAMMATORY CYTOKINES TO DELAY SKIN WOUND HEALING

Thursday, September 13, 2012 / 11:45 - 12:00

Abstract presenter: jean-claude Lecron

Abstract:
CYTO12-1314: CRITICAL ROLE OF TH17 PRO-INFLAMMATORY CYTOKINES TO DELAY SKIN WOUND HEALING
I. Paris 1, S. Charreau 1, E. Guignouard 2, M. Garnier 3, L. Favot-Laforge 4, V. Huguier 1, F.-X. Bernard 5, F. Morel 6, J.-C. Lecron 5*
university of Poitiers/ CHU la milétrie, 2SGS, Université de Poitiers/CHU la Milétrie, university of Poitiers, Université de Poitiers, Poitiers cedex, 5Bioalternatives, Gençay, France

Introduction: The presence of pathogens in skin wound is known to delay wound healing. We previously shown that the proinflammatory cytokines Th17 (IL-22 and IL-17A and F) in combination with TNF, IL-1β and oncostatin M (OSM, IL-6 family) synergistically induce in vitro and in vivo the production of antibacterial peptides and chemokines by human and mouse keratinocytes (Guilloteau et al., J Immunol. 2010), promote proliferation and inhibit their differentiation.

Methods: In order to investigate the presence and the role of these cytokines during wound healing, we developed a C57BL/6 mouse model of cutaneous wound healing, with or without inoculation of wounds by the bacteria S. aureus and P. aeruginosa, able to infect skin.

Results: Wound excision on mice induces the early expression of IL-1β, TNF and OSM, but the expression of IL-22 and IL-17A / F is not detected so far. Bacteria wound inoculation not only increases the expression of IL-1β, TNF and of OSM, but induce a strong expression of IL-22 and IL-17A and F. A same expression pattern is observed in infected human skin wounds. Injection of these five cytokines in the wound edges induce a delayed wound healing similar to that induced by the bacterial mixture. The same experiments in septic conditions but in RAG2-KO mice (deficient in mature lymphocytes) show an accelerated wound healing kinetic, comparable to that of uninfected wild type mice. RAG2-KO skin lesions expressed IL-1β and OSM as wild type mice do, whereas expression of IL-17 and IL-22 is weak or no detectable. It shows that the expression of these cytokines is dependent on the presence of T cells. Finally, supplementation of infected wound edges of RAG2-KO mice by a IL-22, IL-17A and IL-17F mixture delays wound healing at the same level as that of infected wild type mice.

Conclusion: These results demonstrate the synergistic and specific effects of IL-22 and IL-17 in the wound healing delay process, regardless of the presence of bacteria intra se.

References: Guilloteau et al., J Immunol. 2010
Disclosure of Interest: None Declared
Disease-oriented symposium - IL-6 and inflammatory diseases

Room: Room 3&4
Type: Disease oriented symposium
Chairperson: Cem Gabay (Switzerland)

Presentation: Pro- and anti-inflammatory properties of IL-6: consequences for therapeutic blockade

Invited speaker: Stefan Rose-John

Abstract:

CYTO12-1340: THE PRO-INFLAMMATORY ACTIVITIES OF INTERLEUKIN-6 ARE MEDIATED BY TRANS-SIGNALING
S. Rose-John 1,*
UNIVERSITY OF KIEL, Kiel, Germany

Abstract: Cytokines and cytokine receptors exist in membrane bound and soluble form. Membrane bound cytokines need to cleaved in order to become systemically available. While most soluble receptors are antagonists, some are agonists like the soluble IL-6 receptors. In vivo, the IL-6/soluble IL-6R complex stimulates several types of target cells not stimulated by IL-6 alone, since they do not express the membrane bound IL-6R. This process has been named transsignaling [1]. We have shown that soluble gp130 is the natural inhibitor of IL-6/soluble IL-6R complex responses. Recombinant soluble gp130 protein is a molecular tool to discriminate between gp130 responses via membrane bound and soluble IL-6R responses [1]. The soluble IL-6R is mostly generated by proteolysis of the IL-6R transmembrane protein. Shedding of the IL-6R is mediated mainly by the metalloprotease ADAM17, which is also responsible for the cleavage of TNFa and ligands of the EGF-R. Consequently, activation of ADAM17 has different effects on the activation of the immune response as well as on induction of regenerative responses [2,3]. We used neutralizing monoclonal antibodies for global blockade of IL-6 signaling and the sgp130Fc protein for selective blockade of IL-6 trans-signaling in several animal models of human diseases. We could show that inhibition of IL-6 trans-signaling was beneficial in a sepsis model. Also bacterial infection models suggest a different outcome of global blockade of IL-6 as compared to selective IL-6 trans-signaling inhibition [4]. Conclusion: The extent of inflammation is controlled by trans-signaling via the soluble IL-6R. Using the sgp130Fc protein or sgp130Fc transgenic mice we demonstrate in several chronic inflammatory diseases and cancers including inflammatory bowel disease, peritonitis, rheumatoid arthritis, atherosclerosis, colon cancer, ovarian cancer and pancreatic cancer, that IL-6 trans-signaling via the soluble IL-6R is a crucial step in the development and the progression of the disease. Therefore, sgp130Fc is a novel therapeutic agent for the treatment of chronic inflammatory diseases and cancer [5-7].
Disclosure of Interest: None Declared

Presentation: Lessons from the clinical application of IL-6 signal blockade in inflammatory diseases

Thursday, September 13, 2012 / 11:00 - 11:30

Invited speaker: Patricia Woo (United Kingdom)

Presentation: UNEXPECTED COMPLEX STAT3 ACTIVATION ROUTES AND LACK OF NEGATIVE FEEDBACK INHIBITION BY SUPPRESOR OF CYTOKINE SIGNALING (SOCS) PROTEINS IN INTERLEUKIN 23 SIGNAL TRANSDUCTION

Thursday, September 13, 2012 / 11:30 - 11:45

Abstract presenter: Doreen M. Floss

Abstract:

CYTO12-1212: UNEXPECTED COMPLEX STAT3 ACTIVATION ROUTES AND LACK OF NEGATIVE FEEDBACK INHIBITION BY SUPPRESOR OF CYTOKINE SIGNALING (SOCS) PROTEINS IN INTERLEUKIN 23 SIGNAL TRANSDUCTION

D. M. Floss 1, S. Mrotzek 1, J. Schröder 1, S. Rose-John 2, J. Scheller 1
Heinrich-Heine-University, Düsseldorf, Christian-Albrechts-University, Kiel, Germany

Introduction: Signaling of Interleukin 23 (IL-23) via the IL-23 receptor (IL-23R) and the IL-12 receptor beta 1 (IL-12Rβ1) controls innate and adaptive immune responses, and is involved in the proliferation of T h 17 cells. Activation of STAT3 appears to be the major signaling pathway of IL-23, and putative STAT binding sites were predicted in the IL-23R but not in the IL-12Rβ1 chain.

Methods: To elucidate the IL-23 signaling pathway in vitro we generated various expression constructs with amino acid mutations using site-directed mutagensis and deletions within the intracellular domain (ICD) of the murine and human IL-23R. Proliferation of stably transduced Ba/F3-gp130 cells expressing the IL-12Rβ1 and wild-type IL-23R or the mutated/truncated IL-23R variants has been investigated. Phosphorylation and activation of STAT3 was determined by Western blot and FACS analysis.

Results: We showed that the predicted STAT binding sites ((p)YXXQ; Y504, Y626 in murine IL 23R and Y484, Y611 in human IL-23R) mediated STAT3 activation albeit with variable efficiency. Furthermore, we identified two uncommon STAT3 binding/activation sites within the IL-23R. Firstly, the murine IL-23R carried a YPNFQ (Y542) sequence, which acts as an additional activation site of STAT3. Secondly, we identified a phosphotyrosine-independent STAT3 binding motif within the murine and human IL-23R, indicating that pre-association of STAT3 with the IL-23R is required for IL-23 signal transduction. A fourth predicted site, Y416 in murine and Y399 in human IL-23R, was responsible for the activation of the MAPK pathway. In contrast to IL-6-induced short-term STAT3 phosphorylation, cellular activation by IL-23, resulted in long-term STAT3 phosphorylation, indicating that the IL-23R is not a target of negative feedback inhibition by suppressor of cytokine signaling (SOCS) proteins.

Conclusion: In summary, the uncommon STAT3 activation routes in combination with the lack of negative feedback inhibition by SOCS proteins of the IL-23R might explain why IL-6 is only a short-term differentiation factor, whereas IL-23 is a long-term proliferation factor of T h 17 cells.

Disclosure of Interest: None Declared

Presentation: INHIBITION OF THE JAK/STAT PATHWAY PROTECTS AGAINST NEUROINFLAMMATION

Thursday, September 13, 2012 / 11:45 - 12:00

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Abstract presenter: Etty Benveniste

Abstract:
CYTO12-1092: INHIBITION OF THE JAK/STAT PATHWAY PROTECTS AGAINST NEUROINFLAMMATION
E. Benveniste 1,*, H. Qin 1, Y. Liu 1
1UNIV. OF ALABAMA AT BIRMINGHAM, Birmingham, United States

Introduction: Suppressor Of Cytokine Signaling (SOCS) proteins are feedback inhibitors of the JAK/STAT pathway. SOCS3 has a crucial role in inhibiting STAT activation, cytokine signaling and inflammatory gene expression in macrophages/microglia.

Methods: To determine the role of SOCS3 in myeloid cells in neuroinflammation, mice with conditional SOCS3 deletion in myeloid cells (LysMCre-SOCS3fl/fl) were tested for experimental autoimmune encephalomyelitis (EAE).

Results: The myeloid specific SOCS3-deficient mice are vulnerable to MOG-induced EAE, with a severe, non-resolving atypical form of disease. In vivo, enhanced infiltration of inflammatory cells and demyelination is prominent in the cerebellum of myeloid specific SOCS3-deficient mice, as is enhanced STAT3/4 signaling and expression of inflammatory cytokines/chemokines, and an immune response dominated by Th1 and Th17 cells. In vitro, SOCS3-deficient macrophages exhibit heightened STAT3 activation, and are polarized toward the classical M1 phenotype. SOCS3-deficient M1 macrophages provide the microenvironment to polarize Th1 and Th17 cells and induce neuronal death. Furthermore, adoptive transfer of anti-inflammatory M2 macrophages into myeloid SOCS3-deficient mice leads to delayed onset and reduced severity of atypical EAE by decreasing STAT3 activation, Th1/Th17 cells and proinflammatory mediators in the cerebellum. These findings indicate that myeloid cell SOCS3 provides protection from EAE through deactivation of neuroinflammatory responses. LysMCre-SOCS3fl/fl mice are also protected from atypical EAE by the administration of a JAK/STAT3 inhibitor, AZD1480. Severity of disease is attenuated, which is associated with a reduction in STAT1/3 activation, less Th1/Th17 cell infiltration in the cerebellum, reductions in infiltrating neutrophils and macrophages, and decreased M1 proinflammatory gene expression. Mice with classical EAE are also protected against disease severity by treatment with AZD1480.

Conclusion: Collectively, these findings indicate the JAK/STAT3 signaling pathway may serve as a therapeutic target for neuroinflammatory diseases.

Disclosure of Interest: None Declared

Poster session II

Thursday, September 13, 2012 / 12:00 - 14:00

Room: Poster area
Type: Poster session
Description: Have an informal face-to-face meeting with researchers, students and colleagues who will be the utmost pleased to share their work and latest innovations with you, through poster presentation. Lunch will be provided.

Have a look in the "Poster" menu on the left for a list of the presented posters.

Parallel session - Cytokines in tumor immunology

Thursday, September 13, 2012 / 14:00 - 16:00

Room: Room 3&4
Type: Parallel session
Chairperson: Scott Durum (United States)

Presentation: CCL2 orchestrates a tolerogenic environment in the spleen of tumor-bearing hosts

Thursday, September 13, 2012 / 14:00 - 14:30
Invited speaker: Vincenzo Bronte (Italy)

Presentation: Inflammatory cytokines in tumor progression

Thursday, September 13, 2012 / 14:30 - 15:00

Invited speaker: Alberto Mantovani (Italy)

Abstract:

CYTO12-1358: THE YIN YANG OF DECOY RECEPTORS IN TUMOR PROGRESSION
A. Mantovani 1*  
University of Milan, Milan, Italy

Abstract: As early as in the 19th century it was perceived that cancer is linked to inflammation. This perception has waned for a long time. Recent years have seen a renaissance of the inflammation-cancer connection stemming from different lines of work and leading to a generally accepted paradigm (1-4). Recent efforts have shed new light on molecular and cellular pathways linking inflammation and cancer (4). Two pathways link inflammation and cancer. In the intrinsic pathways, activation of different classes of oncogenes drives the expression of inflammation-related programmes which guide the construction of an inflammatory microenvironment. In the extrinsic pathway inflammatory conditions promote cancer development (e.g. colitis-associated cancer of the intestine). Key orchestrators at the intersection of the intrinsic and extrinsic pathway include transcription factors (e.g. NFkB) (5), cytokines (e.g. TNF) and chemokines. Thus, inflammation is a key component of the tumour microenvironment and a target for pharmacologic intervention (e.g. (6)). In a seminal contribution, Hanahan and Weinberg identify six hallmarks of cancer (7). We have surmised that CRI represents a seventh hallmark of cancer (8). Macrophages are key orchestrators of chronic inflammation. They respond to microenvironmental signals with polarized genetic and functional programmes. M1 macrophages which are classically activated by microbial products and interferon-g, are potent effector cells which kill microorganisms and tumors. In contrast, M2 cells, tune inflammation and adaptive immunity; promote cell proliferation by producing growth factors and products of the arginase pathway (ornithine and polyamines); scavenge debris by expressing scavenger receptors; promote angiogenesis, tissue remodeling and repair. M1 and M2 cells represent simplified extremes of a continuum of functional states. Available information suggests that TAM are a prototypic M2 population. M2 polarization of phagocytes sets these cells in a tissue remodeling and repair mode and orchestrate the smouldering and polarized chronic inflammation associated to established neoplasia. Recent studies have begun to address the central issue of the relationship between genetic events causing cancer and activation of protumor inflammatory reactions. Rearrangement of the RET oncogene (RET/PTC) is a frequent, causative and sufficient event in papillary carcinoma of the thyroid. It was recently observed that RET/PTC activates a proinflammatory genetic programme in primary human thyrocytes, including in particular chemokines and chemokine receptors. These molecules are also expressed in vivo and more so in metastatic tumors. These results highlight a direct connection between an early, causative and sufficient oncogene rearrangement and activation of a proinflammatory programme in a human tumor.

Therapeutic targeting of cancer promoting inflammatory reactions is in its infancy, and its development is crucially dependent on defining the underlying cellular and molecular mechanisms in relevant systems. Chemokines are prime targets for interfering with tumor promotion by inflammatory reactions. Ongoing efforts along this line are encouraging. Evidence will be presented that targeting TAM is beneficial in patients.

Disclosure of Interest: None Declared

Presentation: A GRIM PICTURE OF CYTOKINE-INDUCED TUMOR SUPPRESSION

Thursday, September 13, 2012 / 15:00 - 15:15

Abstract presenter: Dhan V. Kalvakolanu
Abstract:
**CYTO12-1086: A GRIM PICTURE OF CYTOKINE-INDUCED TUMOR SUPPRESSION**
D. V. Kalvakolanu 1, S. Kalakonda 2, S. C. Nallar 2, D. J. Lindner 3
UNIVERSITY OF MARYLAND SCHOOL OF MEDICINE, Cleveland Clinic Foundation, Baltimore, United States

**Introduction:** IFNs potently suppress tumor growth which is synergistically enhanced using cell differentiating agents such as retinoic acid (RA). Using a genome-wide knockdown screen, we have identified several novel tumor suppressive gene products regulated IFN/RA called, the Gene(s) associated with Retinoid-Interferon induced Mortality. Here, we will discuss the tumor suppressive properties of GRIM-19, a novel gene discovered in this screen.

**Methods:** Genome-wide knock down screening for tumor suppressors, Protein: protein interactions, Oncogene-induced cytoskeletal protein modification, tumor growth and metastases in vivo.

**Results:** One of the novel genes isolated in the screen, GRIM-19, binds to STAT3 and inhibits growth-promoting gene transcription. It also promotes cell cycle arrest by interacting with the CDK inhibitor p16 Ink4a, a tumor suppressor. GRIM-19 also blocks oncogene induced cell motility, cytoskeletal remodeling, and tumor formation. We show that expression of GRIM-19 is lost and its gene is mutated in several human cancers. Tumor derived mutant GRIM-19 proteins lost their ability suppress constitutively active STAT3 and v-src oncogene induced cellular transformation and tumor growth and metastasis. In addition, GRIM-19 collaborates with other tumor suppressors like p53 for enforcing growth suppression. For example, disrupting HPV-E6 oncogene and E6AP (an ubiquitin ligase, which promotes the degradation of p53), GRIM-19 promotes the stability of p53 tumor suppressor. These observations show that GRIM-19 regulates multiple transcriptional and non-transcriptional processes for inhibiting tumor growth and collaborate with other tumor suppressor pathways. The biological effects of GRIM-19 gene disruption and relevance to tumor suppression and tumor pathology will be discussed.

**Conclusion:** GRIM-19 is a novel tumor suppressor induced by IFNs. It exerts both transcriptional and non-transcriptional effects to control cell growth. Mutations/loss of GRIM-19 expression promotes tumor growth.

**Disclosure of Interest:** None Declared

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Presentation: **SUPPRESSOR OF CYTOKINE SIGNALING-3 INHIBITS BREAST TUMOR KINASE AND STAT3**

*Thursday, September 13, 2012 / 15:15 - 15:30*

**Abstract presenter:** Nancy Reich (United States)

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Abstract:
**CYTO12-1174: SUPPRESSOR OF CYTOKINE SIGNALING-3 INHIBITS BREAST TUMOR KINASE AND STAT3**
Y. Gao 1, V. Cimica 1, N. Reich 1, 2
Stony Brook University, Stony Brook, New York, United States

**Introduction:** The proteins of the SOCS family are best characterized as negative regulators of cytokine signaling and inhibitors of Janus kinases (JAKs) that are bound to cytokine receptors. We describe the distinct ability of SOCS3 to inhibit the activity of breast tumor kinase (Brk), and evaluate the mechanisms by which SOCS3 suppresses Brk. Breast tumor kinase (Brk) is a non-receptor tyrosine kinase that is expressed in a majority of breast tumors, but not in normal mammary glands. One of the substrates of Brk is the signal transducer and activator of transcription 3 (STAT3), a transcription factor causally linked to cancer.

**Methods:** Co-immunoprecipitation assays were used to demonstrate SOCS3 binding to Brk, and protein-protein interactions were used to define the domains of Brk required to bind SOCS3. The ability of Brk to tyrosine phosphorylate STAT3 was used as a measure of its activity, and used to evaluate
Presentation: ONCOGENIC IL-7R GAIN-OF-FUNCTION MUTATIONS IN CHILDHOOD T-ALL

Thursday, September 13, 2012 / 15:30 - 15:45

Abstract presenter: Scott Durum (United States)

Abstract:

CYTO12-1313: ONCOGENIC IL-7R GAIN-OF-FUNCTION MUTATIONS IN CHILDHOOD T-ALL


1National Cancer Institute, NIH, 2National Cancer Institute, Frederick, 3Columbia University, New York, United States, 4Centro Infantil Boldrin, 5Centro Nacional de Pesquisa, Campinas, Brazil, 6Universidade de Lisboa, Lisbon, Portugal, 7Erasmus Medical Center, Rotterdam, Netherlands, 8Universidad Autonoma de Madrid, Madrid, Spain, 9University Medical Center Hamburg, Hamburg, Germany

Introduction: T-cell acute lymphoblastic leukemia (T-ALL) is an aggressive hematological malignancy resulting from leukemic transformation of T-cell progenitors in the thymus. It accounts for approximately 15% of ALL cases in childhood and 20-25% in adults and is a leading cause of death in children. IL-7 and its receptor (IL-7R) play a critical role in normal T-cell development and homeostasis.

Methods: The IL-7R gene was sequenced in T-ALL from three cohorts.

Results: Mutations in IL-7R were identified in 9% of pediatric T-ALL patients. These mutations usually involved insertions of three amino acids including cysteine and proline in the extracellular juxtamembrane region. WT or mutant forms of the human IL-7R (hIL-7R) from patients were retrovirally transfected into an IL-7-dependent murine thymic cell line D1. Mutant hIL-7Rs induced ligand-independent activation of the Jak-Stat and PI3K pathways, cell survival and proliferation. Notably, mutant hIL-7R-expressing D1 cells induced subcutaneous tumors in Rag1−/− mice, with substantial infiltration into various organs that are normally affected in advanced stages of T-ALL, such as bone marrow, liver, lymph nodes and spleen. Further functional assays revealed that mutant hIL-7Rs constitutive signaling required homodimerization via cysteines in the inserted sequences and downstream Jak1 activation, and was IL-7, gc and Jak3-independent.

Conclusion: Our findings indicate that hIL-7R mutational activation drives T-ALL leukemogenesis and implicate IL-7R and Jak1 as therapeutic targets in T-ALL.

Disclosure of Interest: None Declared

Presentation: INHIBITION OF P53 ACTIVITY AND PROTEIN STABILITY BY THE KAPOSI’S SARCOMA-ASSOCIATED HERPESVIRUS-ENCODED NUCLEAR ANTIGEN VIRF-3

Thursday, September 13, 2012 / 15:45 - 16:00

Abstract presenter: Barbora Lubyova
Abstract:

CYTO12-1082: INHIBITION OF P53 ACTIVITY AND PROTEIN STABILITY BY THE KAPOSI'S SARCOMA-ASSOCIATED HERPESVIRUS-ENCODED NUCLEAR ANTIGEN VIRF-3
B. Lubyova 1, *; P. Baresova 1
11st Medical Faculty of Charles University, Institute of Immunology and Microbiology, Prague, Czech Republic

Introduction: Kaposi’s sarcoma-associated herpesvirus (KSHV), or human herpesvirus 8 (HHV-8) is the etiologic agent associated with Kaposi’s sarcoma (KS), primary effusion lymphoma (PEL) and multicentric Castleman’s disease (MCD). Similarly to other herpesviruses, KSHV can enter into two modes of infection: latent infection, in which only five viral genes are expressed, and lytic infection. Our research is focused on oncogenic properties of one of these latent KSHV genes - viral interferon regulatory factor-3 (vIRF-3).

Methods: Co-immunoprecipitation and GST pull-down assays to analyze protein-protein interactions, transfections of HEK293, HeLa and BJAB cells using Superfect, Attractene (Qiagen), or nucleofection (Lonza Research); Western blotting with anti-p53 (DO-1), anti-HAUSP, anti-vIRF-3, and anti-acetylK320 specific antibodies.

Results: In vitro studies suggested that vIRF-3 interacts with the key tumor suppressor gene p53. Therefore, we are currently interested in consequences of this association. Using co-immunoprecipitation method we showed that this interaction can occur in vivo as well. Moreover, vIRF-3 down-regulates p53 protein levels in transfected HEK293 cells and participates in p53 degradation in vIRF-3 transfected BJAB cells. A detailed sequence analysis of vIRF-3 protein identified three potential binding sites with HAUSP/USP7 protein, which is a major p53-specific deubiquitinating enzyme. This association was confirmed in vitro (GST pull-down) and in vivo (co-immunoprecipitation). The consequence of direct binding between p53 and HAUSP is deubiquitination and subsequent stabilization of p53 protein (Li et al., 2002). Our current research is focused on further characterization of vIRF-3-p53-HAUSP binding domains association and functional consequences of this interaction. In addition, after treatment with DNA damaging agent etoposide, vIRF-3 expressing cells exhibit lower levels of p53 acetylation at lysine K320 residue.

Conclusion: Our data suggest that vIRF-3 may play dual role in p53 function: i) direct inhibition of p53 transcription activity, and ii) inhibition of p53 deubiquitination resulting in rapid p53 protein degradation. We believe that this comprehensive analysis will unambiguously establish the oncogenic potential of vIRF-3 and provide a new understanding of the mechanism by which virus-encoded vIRF-3 modulates the function of p53 tumor suppressor. This work was supported by the Grant Agency of the Czech Republic (Grant 204/09/0773), Grant Agency of Charles University (250278) and Charles University SVV-2012-264506.


Disclosure of Interest: None Declared

Parallel session - Pattern recognition receptors and ligands
Thursday, September 13, 2012 / 14:00 - 16:00

Room: Room 2
Type: Parallel session
Chairperson: Paul Hertzog (Australia)

Presentation: Metabolic regulation of Toll-like receptor signaling
Thursday, September 13, 2012 / 14:00 - 14:30

Invited speaker: Luke O'Neill (Ireland)

Abstract:

CYTO12-1345: METABOLIC REGULATION OF IL-1BETA TRANSCRIPTION
L. O'Neill 1, *
TRINITY COLLEGE DUBLIN, Dublin, Ireland

Abstract: In the field of inflammation research, the most important advances in the past 10 years has been in the uncovering of multiple pathways involved in innate immunity. The best characterised involve the Toll-like receptors (TLRs) and NOD-like receptors (NLRs). From work on Nlrp3 there has also been a resurgence of interest in the IL1 system as a key driver of inflammation in diseases such as gout and diabetes (both Type I and Type II). Glucose has also been shown to drive IL-1beta production in beta cells in the pancreas. We have uncovered a process whereby glucose metabolism is required for induction of IL-1beta mRNA. We have evidence that this could involve the transcription factor HIF-1alpha which is stabilised by LPS in a process involving induction of succinate. The role of metabolic processes including glycolysis in the regulation of signalling in inflammation is also likely to be important here. The so-called Warburg effect of aerobic glycolysis is a feature of cells activated during inflammation (including Th17 cells) providing an added level of complexity to signalling pathways activated by TLRs during inflammation.

Disclosure of Interest: None Declared

Presentation: Infection-induced host translational blockage inhibits immune responses and epithelial renewal in the Drosophila gut

Thursday, September 13, 2012 / 14:30 - 15:00

Invited speaker: Bruno Lemaitre (Switzerland)

Abstract:

CYTO12-1354: THE DROSOPHILA GUT: A NEW PARADIGM FOR EPITHELIAL IMMUNE RESPONSE
B. Lemaitre 1,*
EPFL-LAUSANNE, Lausanne, Switzerland

Abstract: The gut combines and integrates very different physiological functions required for maintaining the equilibrium of the whole organism. In addition to its role in digestion, it is the main entry route for pathogens, and a reservoir for resident bacteria that must be tolerated. Finally, the intestinal epithelium undergoes a constant renewal required to maintain the integrity of this barrier. However, little is known about how these functions are regulated and coordinated, or what mechanisms are required to ensure gut homeostasis upon exposure to external challenges such as bacterial infection. Using an integrated approach, we are studying the mechanisms that make the gut an efficient and interactive barrier despite its constant interactions with microbes. We also focus our attention on the regulatory mechanisms that restore gut normal function upon challenge with bacteria. Our projects utilize integrated approaches to dissect not only the gut immune response, but also gut homeostasis and physiology in the presence of microbiota, as well as strategies used by entomopathogens to circumvent these defenses. We believe that the fundamental knowledge generated on Drosophila gut immunity will serve as a paradigm of epithelial immune reactivity and have broader impacts on our comprehension of animal defense mechanisms and gut homeostasis.

Disclosure of Interest: None Declared

Presentation: TLR2 PROMOTES TUMORIGENESIS INDEPENDENT OF INFLAMMATION IN STAT3-DRIVEN GASTRIC CANCER

Thursday, September 13, 2012 / 15:00 - 15:15

Abstract presenter: Brendan Jenkins
Introduction: Cancer of the stomach (gastric cancer) is the second most lethal cancer world-wide, and represents a growing number of cancers that are associated with inflammation. While it is accepted that deregulated interactions between gastric microbes (i.e. Helicobacter pylori) and the host innate immune system are likely to be involved in the pathogenesis of gastric inflammation (gastritis) and cancer, the identity of oncogenic inflammatoryimmune regulators in the host gastric mucosa remains obscure. On this note, deregulated activation of cytokine signaling pathways, especially the pro-inflammatory and oncogenic transcription factor signal transducer and activator of transcription (STAT) 3, is implicated in various inflammation-associated cancers, including up to 50% of human gastric cancers. However, the downstream molecular consequences of aberrant STAT3 activation in promoting carcinogenesis remain to be fully elucidated.

Methods: We report here our investigation into the role of Toll-like receptors (TLRs), which are key components of the innate immune system primarily known to trigger an inflammatory response upon pathogen detection, during gastric cancer. For this purpose, we used 2 independent gastritis/gastric cancer mouse models characterized by STAT3 hyper-activation, 1) gp130F/F mice carrying a specific knock-in mutation in the interleukin (IL)-6 cytokine family co-receptor gp130 which abolishes a negative feedback mechanism, and 2) K19-Wnt1/C2mE transgenic mice displaying the simultaneous activation of the cyclooxygenase-2, prostaglandin E2 and Wnt pathways.

Results: Among the TLR family, aberrant gastric STAT3 activation in these mice specifically caused a significant increase in the expression of TLR2. Genetic targeting of TLR2 inhibited gastric tumorigenesis, but not inflammation, characterized by reduced cellular proliferation and survival in the gastric epithelium. Furthermore, bone marrow chimeras revealed that TLR2-expressing immune/inflammatory cells are dispensable for gastric tumorigenesis. Consistent with our mouse data, in human gastric cancer (epithelial) cells we identified that TLR2 is a direct transcriptional target of STAT3, and activating TLR2 using synthetic lipopeptides promoted cell proliferation via multiple TLR signaling cascades. In human gastric cancer, both increased STAT3 pathway activation and TLR2 expression were negatively associated with patient survival.

Conclusion: Normally considered an innate immune/pathogen recognition receptor, our data reveals an unexpected role for TLR2 in gastric tumorigenesis, whereby increased STAT3 activation results in over-expression of TLR2 to promote gastric epithelial cell growth.

Disclosure of Interest: None Declared

Presentation: A BIMOLECULAR FLUORESCENCE COMPLEMENTATION ASSAY TO STUDY PROTEIN INTERACTIONS IN THE RIG-I LIKE RECEPTOR PATHWAY

Thursday, September 13, 2012 / 15:15 - 15:30

Abstract presenter: María T. Sánchez-Aparicio

Introduction: The host innate immune system acts as the first line of defense to prevent viral invasion. Viral products are rapidly detected through several classes of pathogen recognition receptors (PRR),
Abstract presenter: Ping-Hui Tseng

NATIONAL YANG-MING UNIVERSITY, Taipei, Taiwan, China

Introduction: Toll-like receptors (TLRs) play important roles in innate immunity by eliciting inflammation. Upon receptor engagement, activation of IKKs and MAPKs control the production of cytokines. It has been shown that TAK1 is the essential mediator that transmits signal from receptor to downstream effectors. Meanwhile, in our previous study (1), we implied that IKKs activation in TLR4 is independent to TAK1 phosphorylation. Therefore, the molecular mechanisms underlying how MAPKs and IKKs are differentially activated by TAK1 in TLR4 need for further investigation.

Methods: In order to examine the role of TAK1 in MAPKs and IKKs activation, TAK1-silencing cells were stable expressed with TAK1 mutants and stimulated with LPS. Additionally, LC-MS analysis was used to identify novel post-translational modification and binding partners of TAK1.

Results: As previous reports, we found that the production of cytokines in response to LPS was diminished in TAK1-silencing cells. Reconstituting cells with various mutated TAK1, mutants in either

Disclosure of Interest: None Declared
Abstract presenter: Ashley Mansell

Presentation: INFLUENZA A VIRUS PROTEIN PB1-F2 ACTIVATES THE NLRP3 INFLAMMASOME TO INDUCE INFLAMMATION.

Thursday, September 13, 2012 / 15:45 - 16:00

Disclosure of Interest: None Declared

Abstract:

INTRODUCTION: Characteristic of pandemic influenza A virus (IAV) strains is the hyper-inflammatory response associated with infections which can lead to severe clinical symptoms and death. PB1-F2 is a non-structural IAV protein that has gained attention in recent years mainly attributable via its ability to increase pathogenicity by disrupting macrophages. Critical to human health, most avian influenza and human H3N2 viruses carry the information for full-length PB1-F2 protein, whereas human non-pandemic H1N1 IAV strains harbor a truncated version. Importantly, PB1-F2 peptides form protein aggregates and the formation of PB1-F2 amyloid-like fibres are observed in infected cells. There is increasing awareness to the important role of PB1-F2 in enhanced pathogenesis, although the underlying mechanism for its role in inflammation and macrophage dysfunction is not understood.

METHODS: We have elucidated the mechanism of PB1-F2 induced inflammation via the NLRP3-inflammasome using a series of PB1-F2-deficient viral strains and PB1-F2 peptides combined with inflammasome-deficient macrophages.

RESULTS: Mice treated with IAV lacking expression of PB1-F2 display decreased inflammation, neutrophil, macrophage and DC infiltration to the lung. Conversely, mice inoculated with PB1-F2 peptide display increased inflammatory cell infiltration and inflammation. Following on from this, we have found that PB1-F2 peptide induces maturation of IL-1β in human PBMCs, which is reduced by inhibition of phagocytosis and Caspase-1. PB1-F2 also induced IL-1β secretion in murine macrophages that is phagocytosis-dependent. Importantly, cells deficient in caspase-1, ASC and NLRP3 display ablated IL-1β maturation upon challenge with PB1-F2 indicating PB1-F2 induces inflammation via a NLRP3 inflammasome. These findings suggest a critical role for PB1-F2 in inducing the hyperinflammatory state characterised in pandemic IAV infections and its possible role in macrophage dysfunction via inflammasome-mediated pyroptosis.

activation loop (T184 and T187) or kinase domain (D156) led to inhibition of MAPKs. The activation of IKKs was slightly decreased in D156A mutant, but not T (184; 187) A. Furthermore, mutation in phosphorylation sites of TAK1 enhanced the K63-linked ubiquitination. By in vivo ubiquitination assay, we showed that mutation in either K158 or K209 of TAK1 was unable to abolish polyubiquitination completely, but we confirmed that K158 of TAK1 as the critical site in TLR4 pathway for IKK activation and TAK1 phosphorylation that, thereby, activates of MAPKs. The proteomic analysis for binding partners of TAK1 in different modification status was in line with activity assay. Furthermore, we also indentified K562 of TAK1 as a novel ubiquitination site.

CONCLUSION: We identified that ubiquitination in K209 of TAK1 regulates IKKs activation and the phosphorylation of TAK1 itself. The activation of TAK1 is able to transmit signal to activate MAPKs, and, in turn, block the K63-linked ubiquitination of TAK1 in feedback regulation. Our data suggested that TAK1-mediated activation of downstream effectors in TLR4 is carefully regulated by the crosstalk of phosphorylation and ubiquitination.

**Conclusion:** Our findings identify a previously unknown role for PB1-F2-induced inflammation, via recognition by the NLRP3-inflammasome, leading to maturation of IL-1β. Activation of the inflammasome may also explain the observation of PB1-F2-mediated macrophage dysregulation, possibly via inflammasome-mediated pyroptosis. Elucidating mechanisms of the molecular basis for disease severity of emerging influenza viruses, especially those implicated in global outbreaks is essential to develop better treatment regimes to improve the clinical outcomes for patients with severe acute infections.

**Disclosure of Interest:** None Declared

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**Parallel session - MicroRNA and gene regulation**

*Thursday, September 13, 2012 / 16:30 - 18:30*

**Room:** Room 2  
**Type:** Parallel session  
**Chairperson:** Walter Reith (Switzerland)

**Presentation: Chromatin Exchange in Interferon induced Transcription**  
*Thursday, September 13, 2012 / 16:30 - 17:00*

**Invited speaker:** Keiko Ozato (United States)

**Abstract:**

**CYTO12-1339: CHROMATIN EXCHANGE IN INTERFERON INDUCED TRANSCRIPTION**  
K. Ozato 1.*  
NATIONAL INSTITUTES OF HEALTH, Bethesda, United States

**Abstract:** Type I interferons (IFNs) activate the JAK/STAT pathway and stimulate transcription from many IFN stimulated genes (ISGs). This process likely involves destabilization and reorganization of chromatin. However, chromatin events associated with ISG induction is poorly understood. To gain insight into a link between transcription and chromatin regulation, we studied whether IFN stimulation causes exchange of histones relevant to epigenetic regulation. Because the histone H3.3 is implicated in transcription coupled chromatin change, we constructed NIH3T3 cells expressing GFP- H3.3 and examined H3.3 incorporation into ISGs by ChiP in parallel with ISG transcription and transcription factor recruitment.
IFN stimulation led to rapid recruitment of RNA polymerase II and BRD4, an acetyl-histone binding factor to the ISG. This was followed by recruitment of the elongation factor P-TEFb, the pausing complex NELF/DSIF and SPT6. Along with these events, IFN stimulation caused rapid H3.3 accumulation in the ISGs. H3.3 accumulation was greater in the coding region and the gene end than in the promoter region where virtually no H3.3 incorporation was detected. Analysis with a BRD4 specific inhibitor JQ1 showed that H3.3 incorporation depended on BRD4 recruitment and ISG elongation. However, H3.3 incorporation into ISGs continued past ISG elongation, leaving the H3.3 mark on ISGs for at least two cycles of cell division. The mutant GFP-H3.3K36R was not incorporated into ISGs, indicating that methylation of K36 is required for H3.3 incorporation. Finally, H3.3 incorporation was also observed in another activation model, indicating the generality of transcription-induced histone exchange.

We show that IFN stimulation triggers rapid and extensive H3.3 incorporation into ISGs, which is presumably associated with expulsion of the preexisting H3 (H3.1/H3.2). This event required active ISG elongation, suggesting that passage of RNA polymerase II through the ISG gene body destabilizes the architecture of the RNA-DNA nucleosome, necessitating reconstruction of nucleosomes composed of H3.3. Based on the remarkable persistence of the H3.3 mark left on the ISGs long after transcription, we suggest that transcription-induced H3.3 deposition represents an epigenetic mark linked to transcriptional memory.

**Disclosure of Interest:** None Declared

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**Presentation: MicroRNAs regulating gene expression of cytokines**

*Thursday, September 13, 2012 / 17:00 - 17:30*

*Invited speaker: Steffen Gay (Switzerland)*

**Abstract:**

**CYTO12-1335: MICRORNA'S REGULATING GENE EXPRESSION AS NOVEL MARKERS AND THERAPEUTIC MARKERS**

S. Gay 1,*

UNIVERSITY HOSPITAL, Zurich, Switzerland

**Abstract:** The epigenetic regulatory mechanisms involving all biological processes in health and disease include acetylation of histones, methylation of certain CPG islands in promoters, phosphorylation, sumoylation/ubiquination and miRs or microRNAs. All these processes are intimately intertwined. In this regard, microRNAs can be methylated and regulated by specific histone marks. The best example was shown recently in a most comprehensive study performed by Stefan Kuchen et al characterizing the distinct sets of micro RNAs and their controlling histone marks regulating the immune response in mice (1). Our laboratory studies the various epigenetic regulations in rheumatic diseases (2). With respect to microRNAs, which are short RNA sequences comprised of 20-22 nucleotides, bind to
complementary mRNA sequences along with argonaute proteins resulting in translational repression and thereby silencing of gene expression. More than 1000 microRNAs are involved in the modulation of gene expression. For example, we could demonstrate that microRNA 155 is regulated by TNFa, IL-6 and TLR signaling (3). Moreover, based on the fact that microRNA 155 is involved into B and T cell development, we could show that microRNA ko mice do not develop arthritis (4). On the other hand, we could report that microRNA 203 is regulating the expression of IL-6 and that this microRNA is induced by hypomethylation of its promoter (5). On the other hand, IL-6 regulates the microRNA cluster 17/92 in the down regulation of bone morphogenetic protein receptor 2 (BMPR2) in vascular cells during the development of pulmonary hypertension (6). In related studies Matthias Brock in our lab could show that microRNA-18a enhances the interleukin-6-mediated production of the acute-phase proteins fibrinogen and haptoglobin in human hepatocytes (7). These data reveal, for the first time, a microRNA-mediated positive feedback loop of IL-6 signal transduction leading to an enhanced acute-phase response in human hepatocytes. MicroRNAs will be novel diagnostic biological markers and new therapeutic targets.

Disclosure of Interest: None Declared

Presentation: TRIGGERING MRNA STABILITY SWITCH BY A MIRNA INHIBITOR: NORMALIZATION OF ABERRANT CHEMOKINE RESPONSE IN INVASIVE BREAST CANCER

Thursday, September 13, 2012 / 17:30 - 17:45

Abstract presenter: Khalid S. A. Khabar

Abstract:

CYTO12-1090: TRIGGERING MRNA STABILITY SWITCH BY A MIRNA INHIBITOR: NORMALIZATION OF ABERRANT CHEMOKINE RESPONSE IN INVASIVE BREAST CANCER

M. Al-Ghamdi 1, W. Al-Ahmadi 1, N. Al-Souhibani 1, K. S. A. Khabar 1

King Faisal Specialist Hospital and Research Centre, Riyadh, Saudi Arabia

Introduction: Aberrant mRNA stability for a number of genes coding for AU-rich (ARE) mRNAs, including chemokines and growth factors, occurs in many cancer types. The CXCR4-CXCL12 (SDF-1α) axis is among the most deregulated chemokine responses that promote the progression of invasive breast cancer. Normalization of the aberrant CXCR4-CXCL12 response by triggering CXCR4 ARE-mRNA destabilization mediated by the zinc finger mRNA binding protein, tristetraprolin (TTP, ZFP36), is a potential and intriguing approach.

Methods: Several breast cancer cell lines were used for comparison with the MDA-MB-231 cell line as an invasive model of breast cancer. We treated the cells with a cell-permeable miR-29a inhibitor which triggers TTP expression, and monitored mRNA stability changes for key RNA binding proteins using RT-QPCR and reporter assays. RNA-immunoprecipitation was also employed to study the mRNA-protein interactions. Subsequent alterations in protein levels and associated functional attributes, including invasion and migration assays were investigated. Further, clinical data from a public database were used to understand these relationships.

Results: We found that CXCR-4 over-expression in invasive breast cancer is causally linked to deficient TTP expression. TTP deficiency was partly due to the high miR-29a levels that target TTP 3'UTR- this is observed in the highly-invasive MDA-MB-231 cells when compared to other non-invasive or non-tumorigenic cell lines. We also show that CXCR-4 is an mRNA target for TTP but not its zinc finger mutant, C124R, as the wild type was able to bind to CXCR-4 mRNA. TTP deficiency in MDA-MB-231 cells led to increased stability of mRNA coding for the RNA stabilizing protein, HuR, and subsequently HuR protein levels were increased. We demonstrated that HuR is able to bind to and increase CXCR-4 mRNA stability. Thus, the aberrant TTP-HuR balance results in CXCR-4 mRNA stabilization in invasive breast cancer.
To overcome this aberration, we employed a cell-permeable peptide-linked miR-29a inhibitor to promote TTP mRNA upregulation and HuR mRNA down-regulation. Indeed this was the case, which subsequently led to CXCR-4 mRNA destabilization and reduction of CXCR-4 levels. This paralleled a reduction in the invasiveness of the cells and migration towards CXCR4 ligand, CXCL12.

Clinical data showed that TTP-HuR mRNA ratios which reflect ARE-mRNA stability changes are perturbed in invasive breast cancer samples when compared to breast tissues (p<0.001) and associated with CXCR-4 over-expression (p<0.005).

**Conclusion:** We establish that the chemokine receptor CXCR-4 is a novel mRNA target for TTP and HuR, and that its over-expression and breast cancer invasiveness is normalized by the miR29a inhibitor. The study shows that aberrant ARE-mediated mRNA stability in invasive breast cancer can be corrected by switching TTP-HuR balance towards mRNA destabilization in the CXCR4/CXCR12 axis, indicating a potential therapeutic approach.

**Disclosure of Interest:** None Declared

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**Presentation:** NEGATIVE REGULATION OF TOLL LIKE RECEPTOR SIGNALING BY IL-10 DEPENDENT MICRORNAS

**Thursday, September 13, 2012 / 17:45 - 18:00**

**Abstract presenter:** Graziella Curtale

**Abstract:**

**CYTO12-1196: NEGATIVE REGULATION OF TOLL LIKE RECEPTOR SIGNALING BY IL-10 DEPENDENT MICRORNAS**

G. Curtale 1, T. Renzi 2, M. Mirolo 2, F. Bazzoni 3, M. Rossato 3, M. Locati 1

1Istituto clinico humanitas, rozzano, University of Verona, Verona, Italy

**Introduction:** MicroRNAs (miRs) have emerged as important controllers of Toll-like-Receptor (TLR) but their functional role in the inflammatory response remains incompletely understood.

**Methods: Cells.** Human monocytes obtained from healthy donor buffycoats and THP-1 monocytic cell lines were stimulated with 100ng/ml LPS or Pam3CSK4. **Constructs.** 3'UTR of potential target genes were cloned in psiCHECK vector for the evaluation of miR activity by luciferase assay. For the transduction of THP-1 cell line we generated miRNA/lentiviral-based expression vectors (pRRL-cluster) and cytokines levels were evaluated by ELISA assay. **Bioinformatics and statistical analysis.** The IPA software has been used for the study of pathways associated to predicted miR-125a~99b~let-7e targets. Statistical evaluation was determined using the Student t-test or the One-way ANOVA.

**Results:** To identify candidate miRs potentially involved in the response of human monocytes to stimuli of bacterial origin, we previously analyzed miR expression profile of monocytes stimulated for 8h with LPS1. Among the miRs strongly induced by LPS stimulation we identified the cluster of miR-125a, miR-99b and let-7e. We observed a higher expression of miR-125a, miR-99b and let-7e levels at later time points. As IL-10 is a cytokine late-induced during monocyte activation and exert its anti-inflammatory activity through modulation of gene expression program triggered by LPS, we measured the expression levels of these miRs in monocytes stimulated with IL-10, in the presence or not of LPS, showing a potentiating effect of IL-10 on LPS-induced cluster miR-125a~99b~let-7e expression. We identified a putative STAT3 binding site and demonstrated the binding of STAT3 to this site in the condition of IL-10 stimulation. These data were also consistent with the reduction of cluster miR-125a~99b~let-7e expression levels in the presence of blocking antibody against IL-10R and JAK-STAT inhibitors. An in silico analysis was performed to assess the potential role of cluster miR-125a~99b~let-7e in the context of inflammation and strikingly we found that the TLR pathway was potentially regulated by these miRs at multiple steps of the signalling cascade. We validated both the TLR4 and CD14 receptors as direct targets, as well as key adaptor/signalling proteins, in particular
MyD88 and IRAK1 proteins and also most of the pro-inflammatory cytokines and chemokines expressed in THP-1 monocytic cells. Furthermore, the enforced expression of cluster miR-125a~99b~let-7e in LPS stimulated human monocytes led to an extensive down-regulation of pro-inflammatory cytokines.

Conclusion: Altogether, our results identify a class of IL-10-responsive miRs with anti-inflammatory activity in monocytes based on multiple targeting of components of the TLR4 pathway. We provide a mechanistic insight into the role of these miRs in the suppression of the inflammatory role and candidate them as new feedback modulators of LPS response involved in the resolution of inflammation. A broader and deeper understanding of these issues may well lead to novel therapeutic approaches to those diseases shown to be marked by dysregulated inflammatory responses.


Disclosure of Interest: None Declared

Presentation: INDUCIBLE DELETION OF PRDM1 IN ADULT EPIDERMIS CAUSES CHRONIC SKIN INFLAMMATION IN MICE

Thursday, September 13, 2012 / 18:00 - 18:15

Abstract presenter: Kuo-I Lin

Abstract:
CYTO12-1143: INDUCIBLE DELETION OF PRDM1 IN ADULT EPIDERMIS CAUSES CHRONIC SKIN INFLAMMATION IN MICE
K.-I. Lin 1*, M.-F. Chiang 1 on behalf of Academia Sinica, Taipei 115, Taiwan
1GENOMICS RESEARCH CENTER, ACADEMIA SINICA, Taipei, Taiwan, China

Introduction: B lymphocyte–induced maturation protein-1 (Blimp-1) is a transcriptional repressor important for the differentiation and function of several types of immune cells. Because skin serves as a physical barrier and acts as an immune sentinel, we investigated whether Blimp-1 is involved in epidermal immune function.

Methods: Mice carrying epidermal-specific deletion of Prdm1, the gene encoding Blimp-1, in adult stage in an inducible manner were generated for this study.

Results: We first show that Blimp-1 expression is reduced in stimulated mouse keratinocytes. Epidermal-specific deletion of Prdm1 in adult mice caused spontaneously inflamed skin characterized by massive dermal infiltration of neutrophils/macrophages and development of chronic inflammation, leading to increased release of cytokines/chemokines, including G-CSF, and enhanced granulopoiesis in bone marrow. Deletion of Blimp-1 in the epidermis of adult mice also led to sensitized inflammatory reactions in a disease model of contact dermatitis. Systemic increases in G-CSF contributed to the inflammatory responses because neutralization of G-CSF or deletion of the G-CSF gene, Csf3, corrected neutrophilia and partially ameliorated the inflamed skin in Prdm1-deficient mice.

Conclusion: We show a novel function for Blimp-1 acting in repressing cytokine/chemokine genes in restraining steady-state epidermal immune reactions.

Disclosure of Interest: None Declared

Presentation: THE SIGNALING PROPERTIES OF INTERLEUKIN-30 UNDERLINE PLASTICITY AND CROSS-TALK AMONG THE INTERLEUKIN-6 FAMILY OF CYTOKINES

Thursday, September 13, 2012 / 18:15 - 18:30

Abstract presenter: Christoph Garbers

Abstract:
Introduction: The anti-inflammatory Interleukin (IL)-6/IL-12 cytokine family member IL-27 consists of the cytokine Interleukin-30 (IL-30, also referred to as IL-27p28) and the non-signaling α-receptor subunit Epstein-Barr virus induced gene 3 (EBI3). Antigen-presenting cells are the major cellular source of both proteins, and IL-27 has been shown to engage signaling via a heterodimer of the two transmembrane receptors WSX-1 and gp130. Besides this well-established function, several studies suggest that IL-30 has signaling properties on its own. Recently, IL-30 was shown to form a novel cytokine complex with the non-signaling membrane-bound IL-6 receptor α (IL-6R).

Methods: Since free, endogenous IL-30 is not efficiently secreted, we developed a strategy for bacterial expression, purification and renaturation of murine IL-30 (mIL-30) for in vitro and in vivo analysis. Furthermore, we designed a Hyper-cytokine, consisting of the extracellular/soluble part of the IL-6R fused via a peptide linker to IL-30, and expressed the IL-30-sIL-6R fusion protein in mammalian cells.

Results: We show that mIL-30 can induce STAT-dependent proliferation of Ba/F3-gp130 cells expressing murine or human IL-6R, indicating that in contrast to murine IL-6, murine IL-30 possesses no species specificity. Furthermore, we identified the two transmembrane proteins that function as the signal transducing receptors of the IL-30/IL-6R complex. IL-30 was also able to induce IL-30-trans-signaling using the soluble IL-6R (sIL-6R), a characteristic property that was initially described for IL-6 signaling via the sIL-6R. We further show how STAT phosphorylation and proliferation of Ba/F3-gp130 cells induced by IL-30-sIL-6R can be efficiently inhibited. Finally, mIL-30 but not IL-6, was able to induce STAT-dependent proliferation of Ba/F3-gp130 cells without the need of the p28-α receptors IL-6R and EBI3, albeit at higher concentrations of mIL-30 in comparison to IL-30-sIL-6R.

Conclusion: Our study enlarges the spectrum of IL-30-dependent receptor activation pathways and might have major implications for the biological role of Interleukin-30 in vivo.

Disclosure of Interest: None Declared

Plenary session - T-cell subsets and cytokines

Friday, September 14, 2012 / 08:30 - 10:30

Room: Room 2
Type: Plenary session
Chairperson: Sarah Gaffen (United States), Alberto Mantovani (Italy)

Presentation: IL-17 Receptor Signaling

Friday, September 14, 2012 / 08:30 - 09:00

Invited speaker: Sarah Gaffen (United States)

Presentation: STAT5 and STAT3 as modulators of T-cell differentiation

Friday, September 14, 2012 / 09:00 - 09:30

Invited speaker: Warren Leonard (United States)

Presentation: T-cell differentiation and plasticity in the human system

Friday, September 14, 2012 / 09:30 - 10:00
Invited speaker: Federica Sallusto (Switzerland)

**Presentation:** IL-23 regulation of SCA1+ RORgt+ tissue resident T cells  
*Friday, September 14, 2012 / 10:00 - 10:30*

Invited speaker: Dan Cua (United States)

**Disease-oriented symposium - Cytokine and chemokine targeting in autoimmune diseases I**  
*Room: Room 2  
Type: Disease oriented symposium  
Chairperson: Amanda Proudfoot (Switzerland)*

**Presentation:** Type 1 interferons in multiple sclerosis  
*Friday, September 14, 2012 / 11:00 - 11:30*

Invited speaker: Finn Sellebjerg (Denmark)

**Abstract:**

**CYTO12-1341: TYPE 1 INTERFERONS IN MULTIPLE SCLEROSIS**

F. Sellebjerg 1,*,

DANISH MULTIPLE SCLEROSIS CENTER, Copenhagen, Denmark

**Abstract:** The type 1 interferon (IFN) IFN-beta is used as immunomodulatory treatment for patients with relapsing forms of multiple sclerosis (MS). Recent studies do, however, indicate that endogenous type 1 interferons may also have immunoregulatory effects in MS. Patients with higher activity of an endogenous type 1 IFN gene expression signature have higher expression of interleukin (IL)-10, do not show evidence of myelin-reactive T cell activation, and were reported to have lower clinical and magnetic resonance imaging disease activity. Cell sorting experiments have indicated that monocytes is the main cell type expressing IL-10 in response to IFN-beta. Although IL-27 is also strongly induced by IFN-beta, ex vivo studies indicate that in contrast to what is the case in mice, IL-10 is more important than IL-27 for the immunoregulatory effect of IFN-beta in humans. The endogenous type 1 IFN gene expression signature is controlled by interferon response factors (IRF) such as IRF5 and IRF8, which show association with MS in genetic studies. Surprisingly, however, the alleles conferring an increased risk of developing MS are also alleles associated with increased endogenous IFN activity. We suspect that this may reflect that other genes induced by these IRFs are involved in the pathogenesis of MS.

**Disclosure of Interest:** None Declared

**Presentation:** Targeting CCR9 in IBD  
*Friday, September 14, 2012 / 11:30 - 12:00*

Invited speaker: Satish Keshav (United Kingdom)

**Presentation:** LIGAND-INDEPENDENT BLOCKADE OF TLR4 ACTIVATION REPRESENTS A PROMISING STRATEGY TO PROTECT FROM INFLAMMATORY DISEASE PATHOGENESIS IN ISLET-TRANSPLANTATION  
*Friday, September 14, 2012 / 12:00 - 12:15*

Abstract presenter: Bruno Daubeuf
MOUSE AND ITS PUTATIVE ROLE IN INFLAMMATION

Introduction: Dysregulation of Toll-like receptor 4 (TLR4) signaling via numerous exogenous and endogenous ligands appears to play an underlying role in the pathogenesis of multiple inflammatory diseases. Since multiple TLR4 ligands may be upregulated in a single disease condition, blockade of TLR4 activation via a ligand-independent mechanism is an attractive strategy for disease intervention. A ligand-independent anti-TLR4 neutralizing antibody evaluated as a novel strategy for TLR4 neutralization.

Methods: An anti-human TLR4 mAb, NI-0101, was generated and shown to bind to human TLR4 in a region involved in receptor dimerization. Using exogenous, endogenous or chemical ligands, the capacity to block TLR4 activation was evaluated. Subsequently, NI-0101 was tested in islet-induced immune cell activation. Furthermore, the corresponding mouse surrogate antibody was tested in a laboratory model of islet transplantation.

Results: Due to inhibition of TLR4 dimerization, NI-0101 efficiently blocked the activation of TLR4 by different ligands. Consistent with its ligand-independent mechanism of action, a similar inhibitory potency of NI-0101 was observed at different concentrations of the activating ligand. In addition, NI-0101 could block human islet-induced human immune cell activation, further confirming the ability of NI-0101 to block TLR4 activation independently of the ligand. To determine the effects of NI-0101 in vivo, we used the mouse surrogate antibody of NI-0101, 5E3. 5E3 effectively protected grafted islets in a mouse model of islet transplantation.

Conclusion: The therapeutic anti-human TLR4 mAb, NI-0101, has the capacity to interfere with not only LPS but also signaling of TLR4 through endogenous and chemical ligands. When used in an in vitro model of islet-induced immune cell activation, NI-0101 blocked immune cell activation. Furthermore, we demonstrated that anti-TLR4 mAb is efficient in protecting grafted islets in a mouse islet transplantation model. Taken together, these data promote ligand-independent blockade of TLR4 activation as a promising strategy in blocking disease pathogenesis.

Disclosure of Interest: None Declared

PRESENTATION:

EXPRESSION OF THE DECOY TYPE 2 INTERLEUKIN-1 RECEPTOR IN MOUSE AND ITS PUTATIVE ROLE IN INFLAMMATION

Friday, September 14, 2012 / 12:15 - 12:30

Abstract presenter: Praxedis Martin

Abstract:

CYTO12-1133: EXPRESSION OF THE DECOY TYPE 2 INTERLEUKIN-1 RECEPTOR IN MOUSE AND ITS PUTATIVE ROLE IN INFLAMMATION

P. Martin 1,*, G. Palmer 1, S. Vigne 1, C. Lamacchia 1, D. Strebel 1, E. Rodriguez 1, D. Talabot-Ayer 1, C. Gabay 1

University of Geneva, School of Medicine, Geneva, Switzerland

Introduction: The interleukin (IL)-1 system comprises two agonists, IL-1α and IL-1β (IL-1), a specific receptor antagonist (IL-1Ra) and two receptors, type 1 and type 2 IL-1 receptor (IL-1R1, IL-1R2). As opposed to IL-1R1, IL-1R2 is incapable of signaling because it lacks the intracellular TIR-domain, which is required for signal transduction. Thus, IL-1R2 acts as a decoy receptor for IL-1. This system, together with IL-1Ra, regulates IL-1 activity. In vitro data, mainly obtained in human cells, demonstrated that IL-1R2 is expressed by B cells, monocytes and neutrophils and can be
proteolytically cleaved from the plasma membrane and shed as a soluble form to bind circulating IL-1β. However, although anti-inflammatory activity of IL-1R2 has been described in overexpression studies, the expression of endogenous IL-1R2 in the mouse and its contribution to the control of inflammatory responses has been poorly investigated.

**Methods:** The expression of IL-1R2 transcripts in mouse tissues and ex vivo-isolated or in vitro-differentiated immune cells was assessed by qRT-PCR. For further characterization of endogenously expressed IL-1R2 and its in vitro function, we used bone marrow-derived neutrophils (BMN) for qRT-PCR, extracellular fluorescence activated cell sorting (FACS) and immunoblot analyses. In addition, different mouse models of inflammation were investigated to confirm the expression of IL-1R2 in vivo.

**Results:** IL-1R2 mRNA levels are highly upregulated in lung tissue after LPS-administration. Furthermore, mRNA and protein data obtained using ex vivo Gr1+Ly6G+ peripheral blood cells and in vitro differentiated Gr1+Ly6G+ BMN indicated that, like in human, neutrophils are a major source of IL-1R2 in mouse. However, other mouse immune cells, including T and B lymphocytes, macrophages (both M1 and M2) and dendritic cells do not express detectable levels of the decoy receptor on their cell surface. The expression of IL-1R2 on BMN is regulated by the glucocorticoid hydrocortisone, which induces a strong upregulation of IL-1R2 mRNA and protein. In contrast, the expression of IL-1β but not that of IL-1Ra, is decreased by hydrocortisone, supporting its anti-inflammatory role. LPS treatment induces shedding of IL-1R2 from the membrane into the culture supernatant. We also demonstrate that IL-1R2 binds recombinant mouse IL-1β, but not IL-1Ra. Finally, in in vivo models of inflammation, including thioglycollate-induced acute peritonitis, acute lung injury and two models of arthritis, neutrophils infiltrating the site of inflammation express the decoy receptor IL-1R2.

**Conclusion:** These data indicate that the decoy receptor IL-1R2 is mainly expressed on neutrophils among immune cells in the mouse. It can trap IL-1β and is present during inflammatory situations. Taken together, these results suggest that IL-1R2 plays an important role in regulating IL-1 activity in vivo.

**Disclosure of Interest:** None Declared

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**Disease-oriented symposium - Cytokine targeting in autoimmune diseases II**

*Friday, September 14, 2012 / 11:00 - 12:30*

**Room:** Room 3&4  
**Type:** Disease oriented symposium  
**Description:** with the kind support of Merck Serono  
**Chairperson:** Marie Kosco-Vilbois (Switzerland)

**Presentation:** **New insights into regulatory B cell mechanisms of suppression**

*Friday, September 14, 2012 / 11:00 - 11:30*

**Invited speaker:** Claudia Mauri (United Kingdom)

**Abstract:**

**CYTO12-1030: REGULATORY B CELLS IN HEALTHY AND IN PATIENTS WITH SLE**

C. Mauri 1,*  
UNIVERSITY COLLEGE LONDON, London, United Kingdom

**Abstract:** Recent advances have demonstrated the existence of an IL-10-producing B cell subset with regulatory capacity named (Bregs). In mice, Bregs have been shown to restrain the severity of autoimmune disorders and contribute to the development of infection and cancer. In humans, CD19+CD24hiCD38hiCD1dhi B cells have been ascribed with regulatory function. We have shown that this B cell subset inhibits the activation of T helper (Th)1 and Th17 responses whilst supporting the differentiation of FoxP3+ Tregs. We have shown that, in addition to regulating the differentiation of CD4+ T cells, Bregs are essential for the maintenance of CD1d-restricted invariant natural killer T (iNKT)
cells in healthy individuals but fail to exert the same effect in SLE patients. iNKT cells play a potent immune-regulatory role functioning in both innate and adaptive immunity. Defective B cell-mediated stimulation of iNKT cells in SLE was associated with rapid CD1d recycling leading to reduced CD1d surface expression on B cells from SLE patients, a defect that could be recapitulated in B cells from healthy individuals after simultaneous stimulation with interferon-α (IFN-α) and anti-immunoglobulin (lg). iNKT cell homeostasis was restored in SLE patients responding to B cell depletion therapy, upon normalization of CD1d levels in repopulated CD19⁺CD24hiCD38hiCD1dhi Bregs.

Disclosure of Interest: None Declared

Presentation: Atacicept and SLE: challenges in translational medicine and research
Friday, September 14, 2012 / 11:30 - 12:00

Invited speaker: Henry Hess (Germany)

Abstract:
CYTO12-1349: ATACICEPT: PHASE 2 DEVELOPMENT CANDIDATE FOR THE THERAPY OF SYSTEMIC AUTOIMMUNE SYNDROME
H. Hess 1*
MERCK KGAA, Darmstadt, Germany

Abstract: Atacicept is a novel immunomodulator with B cell targeting properties and currently the advanced clinical development. The primary disease indication for atacicept is SLE, a systemic autoimmune disease with a major involvement of B cells in its etiopathogenesis. Atacicept is a fusion protein comprised of the extracellular domain of the TNF receptor superfamily member TACI (transmembrane activator and calcium-modulator and cyclophilin ligand interactor) and the Fc portion of human IgG1. TACI is expressed on mature B lymphocytes and first detectable on T1 transitional B cells. Expression increases in marginal zone B cells and is finally down-regulated at the plasma cell stage. Two other TNF homologs, BlyS (B lymphocyte stimulator, a.k.a. “BAFF”, or B cell activating factor of the TNF family) and APRIL (A proliferation-inducing ligand), bind with high affinity and specificity to TACI. BlyS enhances the survival and proliferation of transitional B cells. Aside from direct effects on B cells, BlyS and APRIL as well as homo and hetero oligomers are part of the fascinating complexity of immune cell regulation that we are just teetering on the brink of understanding. Despite a well-defined scientific rationale and convincing pre-clinical efficacy profiles, clinical development of atacicept in various indications has passed turbulent times. This presentation is a synopsis of B cell survival factors in health and disease and describing clinical development paths of a molecule that significantly impacts on B cells homeostasis and perhaps beyond.

Disclosure of Interest: None Declared

Presentation: IL-33 AND ST2 KO MICE DISPLAY DIFFERENT PHENOTYPES IN K/BXN SERUM TRANSFER-INDUCED ARTHRITIS
Friday, September 14, 2012 / 12:00 - 12:15

Abstract presenter: Gaby Palmer

Abstract:
CYTO12-1123: IL-33 AND ST2 KO MICE DISPLAY DIFFERENT PHENOTYPES IN K/BXN SERUM TRANSFER-INDUCED ARTHRITIS
P. Martin 1, D. Talabot-Ayer 1, C. A. Seemayer 2, S. Vigne 1, C. Lamacchia 1, E. Rodriguez 1, D. Strebel 1, V. Bochet 1, D. E. Smith 3, C. Gabay 1, G. Palmer 1
University of Geneva School of Medicine, Geneva, 2Novartis Pharma AG, Basel, Switzerland, Amgen Inc, Seattle, WA, United States
Introduction: IL-33 is the most recently described member of the IL-1 family of cytokines. Expression studies and data obtained with ST2 KO mice, ST2 blockade and recombinant IL-33 have suggested implication of the IL-33/ST2 axis in the pathogenesis of human and mouse arthritis. In the present study we investigated the role of endogenous IL-33 in K/BxN serum transfer-induced arthritis by using IL-33 KO mice and compared the results to those obtained using ST2 KO mice.

Methods: Arthritis was induced in IL-33 KO and ST2 KO mice, backcrossed to the C57BL/6 background, or in WT C57BL/6 control mice, by injection of arthritisogenic K/BxN serum or purified total IgG. The development of arthritis was followed by clinical scoring of the paws and confirmed by histological evaluation. Cytokine mRNA expression was assessed by RT-qPCR. Cytokine protein levels were monitored by ELISA or Milliplex.

Results: IL-33 mRNA and protein expression in synovial tissues, as well as circulating IL-33 levels, were similar in arthritic WT and ST2 KO mice, and absent in IL-33 KO mice. The incidence and severity of K/BxN serum transfer-induced arthritis was identical in IL-33 KO and WT mice, while disease development was significantly reduced in ST2 KO mice. Similar data were obtained when injecting purified arthriticogenic total IgG instead of complete K/BxN serum, excluding a potential contribution of IL-33 contained in the serum of donor mice to explain this difference.

Conclusion: IL-33 is expressed locally in the inflamed synovium and is detected in the circulation during serum transfer-induced arthritis. However IL-33 KO mice displayed identical arthritis development and severity as compared to WT controls. In contrast, disease severity was reduced in ST2 KO mice. The reasons for the difference between IL-33 KO and ST2 KO mice, which might suggest IL-33 independent effects of ST2, or reveal the existence of confounding variables in these KO strains, are currently under investigation.

Disclosure of Interest: None Declared

Presentation: IL-17-PRODUCING GAMMA-DELTA T CELLS ARE CRUCIAL FOR THE DEVELOPMENT OF AUTOIMMUNE ARTHRITIS IN IL-1 RECEPTOR ANTAGONIST-DEFICIENT MICE

Friday, September 14, 2012 / 12:15 - 12:30

Abstract presenter: Aoi Akitsu

Abstract:

Introduction: A high proportion of IL-17-producing gamma-delta T (gd17) cells was detected in joints of Il1rm−/− mice, a model of rheumatoid arthritis, whose development depends on IL-17 and T cells. However, their pathogenic roles are not well understood.

Methods: We assessed the effect of gdT cell or CD4+ T cell depletion in Il1rm−/− mice using a monoclonal antibodies. Then, we examined the pathogenic activity of gd17 cells by adoptive transfer.

Results: To clarify the roles of gdT cells and CD4+ T cells in the development of arthritis, gdT cells or CD4+ T cells were depleted in Il1rm−/− mice using antibodies. The development of disease was suppressed in both cases, suggesting both gdT cells and CD4+ T cells were involved in the pathogenesis. Then, the pathogenic role of gd17 cells in the absence of Th17 cells was examined. We generated mice with gd17 cells, but without Th17 cells, by adoptively transferring Il17−/−Il1rm−/−CD4+ T cells into nu/nu mice in which gd17 cells are present. We found that these mice still developed arthritis and that only gdT cells produced IL-17. To corroborate that the development of arthritis in this transfer...
system is dependent on IL-17, we adoptively transferred Il17<sup>-/-</sup>Il1m<sup>+</sup>-CD4<sup>+</sup> T cells into Il17<sup>-/-</sup>-nu/nu mice. The development of arthritis was significantly suppressed in Il17<sup>-/-</sup>-nu/nu mice transferred with Il17<sup>-/-</sup>Il1m<sup>+</sup>-CD4<sup>+</sup> T cells compared with Il17<sup>-/-</sup>-nu/nu mice transferred with Il17<sup>-/-</sup>Il1m<sup>+</sup>-CD4<sup>+</sup> T cells, suggesting that extrathympic gd17 cells are also important for the development of arthritis. Interestingly, Il1m<sup>+</sup> mice on the nu/nu mouse background, in which only gd17 cells but not thymus-derived T cells are present, also developed arthritis. Thus, gd17 cells alone can induce arthritis without involvement of CD4<sup>+</sup> T cells only in Il1m<sup>+</sup> background mice in which excess IL-1 signaling is introduced. In contrast, a combination of CD4<sup>+</sup> T cells and gd17 cells was required for the development of arthritis when scid/scid mice were used as recipients. These observations suggest that gd17 cells are required for the amplification of inflammation and CD4<sup>+</sup> T cells direct the tissue specificity.

**Conclusion:** These results indicate that gdT cell-derived IL-17 plays an important role in the pathogenesis of arthritis in Il1m<sup>+</sup> mice.

**Disclosure of Interest:** None Declared

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**Closing ceremony**

*Friday, September 14, 2012 / 16:00 - 16:30*

**Room:** Room 2  
**Type:** Plenary session

**Parallel session - Microbiome and immunity**

*Friday, September 14, 2012 / 14:00 - 16:00*

**Room:** Room 2  
**Type:** Parallel session  
**Chairperson:** Yolande Chvatchko (Switzerland)

**Presentation:** Dichotomous role of innate cytokines in gut homeostasis and inflammation  
*Friday, September 14, 2012 / 14:00 - 14:30*

**Invited speaker:** Fabio Cominelli (United States)

**Presentation:** Networks of the immune response that ensures mutualism with commensal intestinal microbes  
*Friday, September 14, 2012 / 14:30 - 15:00*

**Invited speaker:** Andrew Macpherson (Switzerland)

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**Abstract:**  
**CYTO12-1035: INNATE AND ADAPTIVE IMMUNITY IN HOST-MICROBIOTA MUTUALISM**  
A. Macpherson<sup>1</sup>  
UNIVERSITY HOSPITAL INSELSPIITAL, Bern, Switzerland

**Abstract:** The mammalian intestine harbours one of the most dense microbial communities on the planet. Experiments that compare germ-free and colonised mice show that the mucosal immune system is highly adapted to the presence of the commensal microbiota. To induce mucosal immune responses to commensal intestinal bacteria, small numbers of commensals are sampled by dendritic cells (DC) at the epithelial surface. These locally induce IgA B cells through T-dependent and T-independent pathways, but they do not penetrate beyond the mesenteric lymph nodes to reach systemic secondary lymphoid structures. Experiments where colonisation of the intestine has been uncoupled from mucosal immune induction with live commensals show that the resulting response is very long-lived unless another response is induced by stimulation with a different commensal microbe. Adaptive immunity in pathogen-free experimental mice is normally ignorant of intestinal commensals. Nevertheless, there is a threshold set by innate immunity, which can strengthen the epithelial barrier.
and allow clearance of small numbers of commensals that escape the permeability barrier or are released by 'commensal loaded' DC. Defective innate clearance is compensated functionally by increased systemic adaptive responses to intestinal commensals, showing a continuum between innate and adaptive immunity.

**Disclosure of Interest:** None Declared

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**Presentation:** DIFFERENTIAL ROLES FOR IL-23 AND IL-17 IN INFLAMMATORY BOWEL DISEASE

*Friday, September 14, 2012 / 15:00 - 15:15*

**Abstract presenter:** Jennifer E. Towne

**Abstract:**

**CYTO12-1260: DIFFERENTIAL ROLES FOR IL-23 AND IL-17 IN INFLAMMATORY BOWEL DISEASE**

J. E. Towne ¹, J. R. Maxwell ¹, Y. Zhang ¹, W. A. Brown ¹, J. B. Rottman ², A. L. Budelsky ¹  
Amgen Inc., Seattle, Amgen Inc., Boston, United States

**Introduction:** Th17 cells and their signature cytokine IL-17A have been implicated in a number of autoimmune diseases including inflammatory bowel disease (IBD). IL-23 is a closely related cytokine to IL-12 in that it shares both a ligand and a receptor subunit. IL-23 drives expression of IL-17A and IL-17F in a number of cell types including Th17 cells. IL-23, IL-17A and IL-17F expression are elevated in IBD and SNPs in the IL-23R show strong association with Crohn's disease and ulcerative colitis.

**Methods:** To examine the role of these cytokines in the gut, we compared inhibition of IL-23p19 and IL-17RA in the *Helicobacter bilis*-infected mdr1a-/- mouse model of colitis.

**Results:** Inhibition of IL-23 alone provided strong efficacy comparable to that seen with an IL-12/23p40 dual inhibitor. Surprisingly, IL-17RA antagonism did not protect mice from disease, and in fact led to acute disease exacerbation. IL-17RA is a necessary receptor for multiple IL-17 family cytokines including IL-17A, IL-17F and IL-25. To better understand which IL-17 family cytokines drive inflammation through IL-17RA in the gut, we compared inhibition of IL-17RA with inhibition of IL-17A, IL-17F or IL-25. IL-17A inhibition resulted in disease exacerbation similar to that seen with inhibition of IL-17RA, while inhibition of the other IL-17 family cytokines had no effect. Subsequent studies found that IL-17RA inhibition did not exacerbate disease in uninfected mdr1a-/- mice. The dependence on the presence of *H. bilis* for the exacerbation suggests either a role for IL-17A in maintenance of barrier integrity or in control of pathogens in the gut. In order to further investigate the mechanisms underlying disease exacerbation in anti-IL-17RA treated *H. bilis*-infected mdr1a-/- mice, we examined gene expression changes in the gut. IL-23 inhibition led to decreased expression of a number of cytokines and chemokines in the gut while IL-17RA or IL-17A inhibition led to increased expression of many genes including IFNγ, TNFα, IL-6 and a number of chemokines. To determine whether an increase in IFNγ was key to the anti-IL-17RA mediated exacerbation, we treated mice with anti-IFNγ along with anti-IL-17RA. Inhibition of IFNγ alone was highly efficacious; however, IFNγ blockade did not affect disease in the presence of anti-IL-17RA. These data suggest that pathways other than IFNγ may be driving anti-IL-17RA mediated disease exacerbation in this mouse model of colitis and the exacerbation is not likely due to a shift from Th17 cells to Th1 cells.

**Conclusion:** The data in this *H. bilis*-infected mdr1a-/- mouse colitis model are similar to what is emerging from Crohn's disease clinical trials: IL-12/23p40 inhibitors are showing promise while inhibition of IL-17A with secukinumab led to exacerbation in a subset of patients. The secukinumab data in Crohn's disease patients are in contrast to the data in psoriasis patients where inhibition of IL-17A, IL-17RA or IL-23 have shown promising efficacy. Together the emerging clinical data suggest that the role of Th17 cells and IL-17A in colitis is different from other inflammatory diseases and that IL-23 and IL-17 biology are distinct in the gut.

**Abstract:**

**CYTO12-1025: ROLE OF HUMAN TOLEROGENIC DENDRITIC CELL DERIVED IL-35 IN REGULATING T CELL RESPONSES**

K. Dixon 1,*, S. van der Kooij 1, N. Schlagwein 1, D. A. Vignali 2, C. van Kooten 1

1Leiden University Medical Centre, Leiden, Netherlands, St. Jude Children's Research Hospital, Memphis, United States

**Introduction:** IL-35 is a novel cytokine of the IL-12 family, existing as a heterodimer of IL-12p35 and EBV-induced gene 3 (Ebi3). IL-35 is produced by natural and inducible regulatory T cells in humans and mice and seems required for optimal suppression. Dexamethasone (Dex) and 1, 25-dihydroxyvitamin D3 (D3) have previously been shown to inhibit IL-12 production in activated dendritic cells (DC) and suppress allogenic T cell responses. Tolerogenic DCs are potentially useful to specifically alleviate unwanted immune responses and induce immune tolerance in transplantation and autoimmunity. In this study, we investigated the regulation of production of various IL-12 family members in human tolerogenic DC (tDC), with a main focus on IL-35.

**Methods:** Monocytes were isolated from human PBMC and cultured for 6 days in the presence of IL-4 and GM-CSF to obtain immature DC. Tolerogenic dendritic cells were generated using the same culture conditions but with the addition of Dexamethasone for DexDC, or Dexamethasone with 1, 25-dihydroxyvitamin D3 for D3Dex. RT-PCR quantified IL-12A, IL-12B, IL-27A, IL-23A, EBi3 and IL-10. ELISA was used to measure IL-12p40, IL-12p70, IL-10 and IFN-γ in cellular supernatants. Intracellular expression of IL-12p35, Ebi3 and IL-12p40 was measured by flow cytometry. Expression of IL-12p35 and Ebi3 was evaluated using confocal microscopy and western blot. For T cell suppression assays naive CD4+ T cells were isolated from cord blood or PBMCs and stimulated with anti-CD3/28 in the presence or absence of DC derived supernatants. Proliferation was measured by 3H-thymidine incorporation.

**Results:** We demonstrate by Q-PCR that, although tDC completely lack the expression of IL-12p40, they maintain mRNA expression of Ebi3 and IL-12p35. In line with this, tDC do not produce bioactive IL-12p70 or the homodimer IL-12p40. Using intracellular FACS and western blot we show that tDC maintain the protein expression of both Ebi3 and IL-12-p35. Expression of these proteins can be further enhanced upon stimulation with pro-inflammatory cytokines, TLR agonists and CD40 ligation. Functional inhibition studies show that tDC derived supernatants have the ability to suppress T cell proliferation. At present we are performing blocking experiments in tDC supernatants to fully evaluate the extent at which IL-35 contributes to the tolerogenic characteristic these cells possess in regulating T cell responses.

**Conclusion:** Taken together, our results suggest that aside from increased expression of previously described tolerance inducing markers (B7-H1, I L T3 etc) and increased IL-10 production, tDC produce IL-35, providing an additional novel mechanism by which these cells elicit their tolerogenic potential.

**References:**


Disclosure of Interest: None Declared

Presentation: THE INTERFERONSCAPE: SYSTEMS BIOLOGY OF INTERFERON STIMULATED PATHWAYS AND NETWORKS

Friday, September 14, 2012 / 15:30 - 15:45

Abstract presenter: Shamith A. Samarajiwa

Abstract:
CYTO12-1316: THE INTERFERONSCAPE: SYSTEMS BIOLOGY OF INTERFERON STIMULATED PATHWAYS AND NETWORKS
S. A. Samarajiwa 1,2,*, I. Spiteri 2, A. Lynch 1,2, A. Jauhiainen 3, E. Morrissey 1, J. Cairns 1,2, M. Narita 2, S. Tavaré 1,2
University of Cambridge, 2CRUK Cambridge Research Institute, Cambridge, United Kingdom, Karolinska Institutet, Stockholm, Sweden

Introduction: The Interferons (IFNs) are a family of pleiotropic cytokines that mediate anti-microbial, anti-proliferative, anti-tumour, immuno-modulatory and homoeostatic host defence functions. Differential regulation of Interferon Stimulated Genes (ISGs) by IFNs, via multiple signaling pathways and the combinatorial effects of IFN-induced transcription factors, co-regulators, ncRNA and epigenetic modifications underlie these diverse biological functions.

Methods: We have built InterferonScape- a software platform for data-integration, mining and visualization to study IFN mediated ISG regulation at a systems level. This is a centralized systems-immunology resource that integrates ISG datasets into a data warehouse with a multidimensional data analysis capability. Statistical and computational data-mining methods combined with easy to use graphical user interfaces are included to provide custom-built data-visualization capability. This resource is provided as a web 2.0 rich internet application with novel embedded data-mining, search and semantic web technologies.

Results: Using integrative data-mining approaches we previously identified approximately 1800 ISGs in the human genome (Samarajiwa et al., 2009). Very few of these ISGs have been studied systematically, and information on ISG pathways is virtually absent from both public and commercial pathway repositories. We have utilised computational pathway modelling approaches, coupled to natural language text mining and expert manual curation to build a reliable collection of ISG pathways and networks. By integrating both in-house generated and publicly-available gene expression, regulatory genomic and epigenetic datasets in different cell and tissue types and in both normal and disease conditions, we have identified the regulatory elements and promoter modules involved in the regulation of ISG networks.

Conclusion: Using integrative genomic and computational biology approaches we will demonstrate the evolutionary conservation, information flow, connectivity, network topology and regulatory interactions underlying these ISG networks. Understanding the propagation of ISG networks in different conditions will provide a greater insight into IFN biology in health and disease.


Disclosure of Interest: None Declared

Presentation: A NOVEL ROLE OF DECTIN-1 SIGNALING IN PROMOTING INTESTINAL INFLAMMATION

Friday, September 14, 2012 / 15:45 - 16:00
Abstract presenter: Ce Tang

Abstract

CYTO12-1207: A NOVEL ROLE OF DECTIN-1 SIGNALING IN PROMOTING INTESTINAL INFLAMMATION
C. Tang 1,2,*, T. Kamiya 1, M. Kadoki 1, Y. Iwakura 1,2
Research Institute for Biomedical Sciences, Tokyo University of Science, Noda, Japan Science and Technology Agency, Saitama, Japan

Introduction: Dectin-1, which was first reported as a dendritic cell-specific type II C-type lectin family member, is the receptor for β-1,3 or -1,6-linked glucans (β-glucans), an important cell wall components of fungi and yeasts. Dysregulated response of mucosal immune system toward intraluminal bacteria results in the human inflammatory bowel disease (IBD). Beta-glucan is thought to promote the mucosal immunity in intestines, but the roles of Dectin-1 in mucosal immune system are still unknown.

Methods: To investigate the potential role of Dectin-1 in development of IBD, we administrated Decint-1 deficient (clec7a-/-) mice with dextran sulfate sodium (DSS) to induce the acute ulcerative colitis and found that clec7a-/- mice were significantly resistant to DSS-induced colitis compared to wild-type (WT) mice, associated with lower production of TNF-α and reduced numbers of neutrophils and macrophages in lamina propria of colon.

Results: Pre-treatment of dectin-1 antagonist ligand Laminarin could suppress the acute intestinal inflammation induced by DSS. Metagenome analysis using bacterial 16s rRNA genes revealed significantly change of the microflora in small intestine and colon of clec7a-/- mice compared to WT mice, and administration of intestinal bacteria of clec7a-/- mice in SPF condition to germ-free WT mice also showed resistant to DSS-induced colitis.

Conclusion: These new findings identify Dectin-1 as novel factor in promotion of acute colitis by directly inducing proinflammatory cytokines and by regulating the balance of intestinal microbiota. Blockade of Dectin-1 signaling suggests new therapeutic strategies for inflammatory bowel diseases.

Disclosure of Interest: None Declared

Parallel session - Cytokine and interferon signaling

Wednesday, September 12, 2012 / 14:00 - 16:00

Room: Room 3&4
Type: Parallel session
Chairperson: Charles Samuel (United States)

Presentation: Adenosine deaminase (ADAR1) acting on double-stranded RNA and protein kinase PKR dependent on dsRNA as opposing modulators of antiviral innate immunity

Wednesday, September 12, 2012 / 14:00 - 14:30

Invited speaker: Charles Samuel (United States)
Double-stranded RNA (dsRNA) plays a central role in antiviral innate immunity, both as a trigger for the production of interferon (IFN) and also in the actions of IFN. Adenosine deaminases acting on RNA (ADAR) catalyze the C-6 deamination of adenosine (A) to generate inosine (I) in dsRNAs. Because I hydrogen bonds as G with C, not as A with U, A-to-I editing can lead to genetic recoding of RNAs and destabilization of dsRNA structures. The Adar1 gene is IFN inducible and encodes two size forms of ADAR1 protein through alternative promoter utilization; an IFN inducible promoter specifies a p150 protein that is cytoplasmic and nuclear, whereas the constitutive promoters specify a smaller constitutively expressed p110 nuclear protein. Protein kinase PKR also is IFN inducible, is activated by dsRNA, and inhibits translation through eIF-2alpha factor phosphorylation. The roles of ADAR1 p150 and p110, and PKR, during virus replication were examined using human cell clones made stably deficient in ADAR1 or PKR by an RNA interference strategy, and mouse MEF cells genetically deficient in either ADAR1 or 2. Results obtained with wild-type measles virus (MV) and mutants deleted for either C or V expression revealed important roles for both ADAR1 and PKR as modulators of IFN induction and the host response to infection. The effects of ADAR1 deficiency on MV growth, IFN beta gene induction, and virus-induced cytopathic effect generally were opposite to the effects observed for PKR deficiency. The findings indicate that ADAR1 behaves as an anti-apoptotic host factor that, in some instances, is pro-viral. The anti-apoptotic and cell protective effects of ADAR1 correlated with suppression of activation of pro-apoptotic activities exemplified by PKR. Results obtained with mouse cells genetically deficient in adar1 likewise showed a protective role of ADAR1 protein isoforms against virus-induced cytopathic effects. (Supported by NIAID, NIH).

**Disclosure of Interest:** None Declared

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**Presentation:** Signaling pathways that mediate mRNA translation of ISGS and their roles in the induction of IFN responses

*Wednesday, September 12, 2012 / 14:30 - 15:00*

**Invited speaker:** Leonidas Platanias (United States)

**Abstract:**

**CYTO12-1294: SIGNALING PATHWAYS THAT MEDIATE MRNA TRANSLATION OF ISGS AND THEIR ROLES IN THE INDUCTION OF IFN RESPONSES.**

L. Platanias 1/

1/NORTHWESTERN UNIVERSITY, Chicago, United States

**Abstract:** There is accumulating evidence that the mTOR pathway plays key and essential roles in the generation of IFN-responses. Our studies have shown that Type I and II IFN receptors activate mTORC1 complexes, which in turn regulate downstream effector signals essential for initiation of mRNA translation and/or protein expression of interferon stimulated genes (ISGs) whose transcription is regulated by Jak-Stat pathways. In recent studies we have established key and essential roles for mTORC2 complexes in the generation of IFN-responses. The current status of this research area will be reviewed and an update on ongoing efforts to define mTOR-controlled cellular events in the IFN-system and their functional relevance will be provided.

**Disclosure of Interest:** None Declared

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**Presentation:** FINE STRUCTURAL AND FUNCTIONAL CHARACTERIZATION OF A UNIQUE IFNB-IFNAR1 SIGNALING AXIS.

*Wednesday, September 12, 2012 / 15:00 - 15:15*

**Abstract presenter:** Nicole De Weerd
Abstract: CYT012-1148: FINE STRUCTURAL AND FUNCTIONAL CHARACTERIZATION OF A UNIQUE IFNB-IFNAR1 SIGNALING AXIS.
N. De Weerd 1,*, T. Nguyen 1, J. Vivian 2, N. E. Mangan 1, J. A. Gould 1, S. Noppert 1, L. Zaker-Tabrizi 1, T. Beddoe 2, H. H. Reid 2, J. Rossjohn 2, P. J. Hertzog 1
1Monash Institute of Medical Research, 2Monash University, Clayton, Australia

Introduction: Type I interferons (IFNs) are an important family of cytokines which enable the immune system to fight viral infections and cancer, and modulate the immune response. The type I IFNs are unique amongst cytokines since multiple ligands all bind to and signal through the same heterodimeric receptor. Despite a shared receptor complex, discernible differences result from receptor engagement by the different IFN subtypes. Conventionally, activation of IFN signaling complex formation is initiated by ligand binding to the high affinity receptor (Ifnar2) with the subsequent recruitment of the low affinity receptor component (Ifnar1). Ligand-induced cross-linking in this manner results in activation of the canonical Jak/STAT signaling pathway and up-regulation of numerous interferon responsive genes.

Methods: In the current study, we have used protein crystallography to determine the fine structure of IFNβ bound to the extracellular domain (ECD) of Ifnar1. We have also used microarray analysis, RT-PCR and an animal model of sepsis to investigate the consequences of the IFNβ/Ifnar1 interaction.

Results: In the current study we demonstrate that a different functional capability and property of IFNβ is dictated by its ability to interact directly via Ifnar1, independently of Ifnar2. We thus report the crystal structure of IFNβ in complex with the full length, extracellular domain of Ifnar1 and demonstrate unique interaction interfaces between these proteins not previously defined by earlier structural studies of other type I IFN complexes. We also demonstrate that this complex not only occurs in vivo but participates in the transduction of IFNβ signals in mice. Using microarray analysis we have identified a novel IFNβ signaling axis that occurs in the absence of Ifnar2 and that induces the expression of genes encoding chemokines, cytokines and other known interferon stimulated genes. Using a mouse model of sepsis we also show that it is the unique mode of Ifnar1 engagement by IFNβ that leads to lethality in this disease model.

Conclusion: Our results reveal for the first time the unique mechanism of Ifnar receptor engagement by IFNβ and may help to explain the unique functional attributes that IFNβ possesses.

Disclosure of Interest: None Declared

Presentation: ACTIVATION OF THE SYK-CARD9-BCL10-MEDIATED NOVEL INNATE IMMUNE SYSTEM IS CRITICALLY INVOLVED IN DEFENSE AGAINST LEISHMANIA MAJOR INFECTION

Wednesday, September 12, 2012 / 15:15 - 15:30

Abstract presenter: Hiroki Yoshida

Abstract: CYT012-1054: ACTIVATION OF THE SYK-CARD9-BCL10-MEDIATED NOVEL INNATE IMMUNE SYSTEM IS CRITICALLY INVOLVED IN DEFENSE AGAINST LEISHMANIA MAJOR INFECTION
H. Yoshida 1,*, H. Hara 1, M. Nakaya 1, F. Mi-ichi 1, M. Kubota 1
Saga University, Faculty of Medicine, Saga, Japan

Introduction: While Th1-type acquired immunity is important for defense against Leishmania major, it remains elusive how innate immunity is activated in response to L. major infection. ITAM-coupled receptor/Syk/CARD9/Bcl10 signaling, a novel innate immune system, is essential for NF-κB activation and inflammatory cytokine production in response to, for instance, fungal infection.

Methods: To examine the involvement of the CARD9-mediated innate immune system in defense against L. major, CARD9-deficient dendritic cells (DCs) were infected in vitro with L. major and TNF-α production examined. Activation of Syk/CARD9-mediated signaling was also examined. CARD9-
deficient mice were infected in vivo with L. major and susceptibility and local inflammatory responses, as well as Th1 differentiation were examined.

**Results:** TNF-α production by CARD9-deficient DCs in vitro was impaired as compared to wild-type or MyD88-deficient DCs. Involvement of Syk and Bcl-10 was also demonstrated. In vivo, swelling as well as parasite burden in L. major-infected footpads 2 weeks after infection was significantly increased in CARD9-deficient mice as compared to wild-type mice. Consistent with the parasite burden, both L. major-specific Th1 induction and pro-inflammatory cytokine production at an early phase of infection were reduced in CARD9-deficient mice as compared to wild-type mice.

**Conclusion:** Syk-CARD9-Bcl10 signaling is crucial for the activation of innate immunity at the early stage of L. major infection and regulates the subsequent induction of proper Th1 cell responses. This is the first observation that protozoa infection activates the novel innate immune system. To identify the responsible receptors, we screened a series of known ITAM-coupled receptor-Ig fusion proteins for binding to L. major and have found some candidates that may act as innate sensors for L. major.

**Disclosure of Interest:** None Declared

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**Presentation:** IL-17 RECEPTOR ADAPTOR ACT1/CIKS PLAYS AN EVOLUTIONARILY CONSERVED ROLE IN ANTIVIRAL SIGNALLING

*Wednesday, September 12, 2012 / 15:30 - 15:45*

**Abstract presenter:** Grigory Ryzhakov

**Abstract:**

**CYTO12-1151: IL-17 RECEPTOR ADAPTOR ACT1/CIKS PLAYS AN EVOLUTIONARILY CONSERVED ROLE IN ANTIVIRAL SIGNALLING**

G. Ryzhakov 1, K. Blazek 1, C. C.-K. Lai 2, I. A. Udalova 1

Oxford University, Imperial College, London, United Kingdom

**Introduction:** Double stranded RNA-induced antiviral gene expression in mammalian cells depends on activation of Interferon Regulatory Factor 3 (IRF3)1,2. As the expression of the Interleukin-17 receptor adaptor protein Act1 is induced in response to double-stranded RNA, we asked whether Act1 is involved in the IRF3-dependent antiviral signalling.

**Methods:** RNA interference was used to knockdown Act1 expression in primary human fibroblasts to test the protein’s involvement in polyinosinic:polycytidylic acid (poly(I:C))-induced antiviral gene expression, measured by quantitative PCR (qPCR), and IRF3 activation detected with a phospho-IRF3 antibody. Also, transient transfection of human 293 ET cells with expression plasmids, encoding Act1 and other components of antiviral pathways, was used to monitor the activation of IRF3 using an IRF3-specific luciferase-based reporter assay and qPCR. Point mutations and deletions of Act1 were created to map specific regions responsible for its interactions with other components of the antiviral signalling. Zebrafish Act1 was cloned into a mammalian expression vector and compared to its human counterpart in terms of its ability to trigger IRF3 activation (using the above-mentioned reporter assay) and antiviral gene expression measured by qPCR.

**Results:** (1) The siRNA-mediated knockdown of Act1 inhibits antiviral gene expression and IRF3 phosphorylation induced by poly(I:C) stimulation, while the overexpression of Act1 potentiates IRF3-driven antiviral gene expression; (2) Act1 interacts with the components of antiviral signalling pathways, IKKε and IRF3, using distinct domains; (3) Act1-induced IRF3 activation can be blocked specifically by co-expression of a catalytically-inactive mutant of IKKε; (4) mutants of IRF3, either lacking the C-terminus or mutated at the key phosphorylation sites, important for its activation by IKKε, do not support Act1-dependent IRF3 activation; (5) Zebrafish Act1 protein is able to trigger antiviral gene expression in human cells.

**Conclusion:** Act1 is a novel component of antiviral signalling, which functions as a signalling adaptor of IKKε and IRF3. Comparison of mammalian and fish Act1 proteins suggests evolutionary conservation of the antiviral function of Act1 in vertebrates.

Disclosure of Interest: None Declared

Presentation: STRUCTURAL AND MOLECULAR BASIS OF IL-3/GM-CSF/IL-5 RECEPTOR SIGNALLING

Wednesday, September 12, 2012 / 15:45 - 16:00

Abstract presenter: Angel Lopez

Abstract:

CYTO12-1034: STRUCTURAL AND MOLECULAR BASIS OF IL-3/GM-CSF/IL-5 RECEPTOR SIGNALLING
A. Lopez 1,*, T. Hercus 1, B. McClure 1, S. Broughton 2, U. Dhagat 2, T. Nero 2, M. Parker 2
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Introduction: Interleukin (IL)-3, granulocyte-macrophage colony stimulating factor (GM-CSF) and IL-5 are important cytokines that bridge innate and adaptive immunity, and play a significant role in cancer. They control the proliferation and function of myeloid cells and dendritic cells, and are recognised key players in crippling immunopathological conditions such as rheumatoid arthritis and asthma. All three cytokines signal through heterodimeric specific receptors consisting of major binding and ligand-specific α chains, and a signalling subunit βc, which is shared amongst all three receptors. The IL-3, GM-CSF and IL-5 receptors are also themselves important determinants of disease. For example, we have established a functional role for the IL-3 receptor α chain (CD123) in promoting the survival, homing, proliferation and engraftment of acute myeloid leukaemic stem cells using our blocking monoclonal antibody 7G3 in vivo and demonstrated a number of patients in which IL-3 autocrine signalling occurs in vitro in leukaemic stem cell populations (1). In addition, our genetic studies on βc showed that this receptor subunit is required for the development of asthma (2).

Methods: We have used a combination of molecular biology, cell biology, biochemistry, crystallization and computer modelling to obtain 3D structures of cytokine receptors.

Results: To understand how these receptors signal and to help in the rational design and pre-clinical testing of future biologicals and small molecules that can selectively eliminate cancer stem cells or block inflammatory cells requires detailed 3D structural knowledge of druggable targets. Towards this aim we have recently solved the crystal structure of the human GM-CSF receptor ternary complex that revealed a novel mode of cytokine receptor activation involving a higher-order dodecamer complex (3); however, details of some specific receptor: ligand interactions were missing. We have now solved the structure of the binary GM-CSF: GM-CSF receptor α chain complex based on X-ray diffraction data collected to 2.8 Å resolution which reveals for the first time all three extra cellular domains of the GM-CSF α receptor chain. In particular, this new structure suggests a mechanism of binding involving the N-terminal domain that differs from the related IL-3 receptor. Intriguingly, docking of the complete GM-CSF receptor α chain onto the dodecamer complex places the N-terminal domains from adjacent hexamers in close proximity to each other suggesting a direct role in receptor activation. We have now assembled and obtained crystals of IL-3 receptor complexes in solution comparable to those obtained for GM-CSF representing binary (IL-3 receptor α chain:IL-3) and ternary (IL-3 receptor α chain+βc +IL-3 ) complexes. Importantly, we have also crystallised and solved the structure of the IL-3 receptor α chain in complex with an antagonist monoclonal antibody.

Conclusion: This provides unique information on the IL-3 binding site in the IL-3 receptor α chain, and optimisation opportunities for our antagonistic monoclonal antibody towards its more effective use.
Details of these structures and their implications for receptor signalling and the design of antagonists will be discussed.


**Disclosure of Interest:** None Declared

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**Parallel session - IFN regulation of viral pathogenesis**  
*Thursday, September 13, 2012 / 16:30 - 18:30*

**Room:** Room 3&4  
**Type:** Parallel session  
**Chairperson:** Adolfo Garcia-Sastre (United States)

**Presentation: Antiviral actions of the interferon-inducible IFIT proteins**  
*Thursday, September 13, 2012 / 16:30 - 17:00*

**Invited speaker:** Ganes Sen (United States)

**Abstract:**

**CYTO12-1346: ANTIVIRAL ACTIONS OF THE INTERFERON-INDUCIBLE IFIT PROTEINS**  
G. Sen 1,*  
1CLEVELAND CLINIC, Cleveland, United States

**Abstract: Antiviral actions of the interferon-inducible Ifit proteins**

Ganes Sen, Volker Fensterl, Jamie Wetzel

The ISG56/IFIT genes are a multi-member family of interferon-inducible genes; there are four members in human and three in mouse. All IFIT proteins contain multiple TPR motifs which mediate protein-protein interactions. Some, but not all, members inhibit initiation of protein synthesis by binding to the translation initiation factor eIF-3 or by binding to the 5' end of the mRNA. The murine Ifit1 encodes P56, Ifit2 encodes P54 and Ifit3 encodes P49. For investigating the biological functions of the murine Ifit proteins, we have recently generated several knock-out mice. Challenging Ifit1-/- and Ifit2-/- mice with various viruses have revealed their strong, but selective, antiviral properties. Ifit1-mediated host restriction was shown to be evaded by 2'-O-methylation of viral mRNAs. Flavivirus (West Nile Virus), vaccinia virus and coronavirus mutants, that lack 2'-O-methylation of their mRNAs, were growth-restricted in Wt, but not in Ifit1-/-, cells and mice. Further studies revealed that the action of Ifit1 on WNV replication is highly manifested in CNS infection, a deficiency of Ifit1 causing increased neuronal death in infected mice. Ifit2, on the other hand, blocked neuro-pathogenesis caused by intranasal infection with the rhabdovirus, VSV. All Ifit proteins were induced in the CNS of the infected mice, but Ifit1-/- mice were not more susceptible than Wt mice and most of the infected mice survived. In contrast, all Ifit2-/- mice died from neuro-pathogenesis; VSV replicated efficiently in the neurons of these mice. However, there was no effect on pathogenesis by another neuro-tropic virus, EMCV. Moreover, VSV did not replicate efficiently in the liver or the lung of infected Ifit2-/- mice and in vitro, in MEF or primary fetal neurons, the sensitivity of VSV replication to IFN-treatment was similar in Wt and Ifit2-/- cells. These results demonstrated the existence of tissue-, virus- and ISG-specific antiviral actions of interferon.

**Disclosure of Interest:** None Declared

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**Presentation: Positive and negative regulation of the RIG-I antiviral pathway**  
*Thursday, September 13, 2012 / 17:00 - 17:30*

**Invited speaker:** John Hiscott (United States)
Abstract: The cytosolic RIG-I pathway is activated by many RNA viruses - including influenza - via viral RNA replicative intermediates that contain short hairpin dsRNA and 5’ triphosphate structures. We characterized natural and synthetic RIG-I agonists based on sequences from the 5’UTR regions of distinct negative-strand viruses – vesicular stomatitis virus (VSV), Influenza, Rabies, Measles, and Sendai virus - and demonstrated potent stimulation of RIG-I antiviral responses at concentrations in the picomolar range. In human bronchial epithelial A549 cells, 5’pppRNA induced IRF3 phosphorylation and dimerization, STAT1 Tyr701 phosphorylation, as well as a >100-fold increase in the transcription of interferon stimulated genes (ISGs) and genes involved in inflammation. The magnitude and duration of ISG and inflammatory gene expression was evaluated by gene expression profiling, where 5’pppRNA triggered a sustained and diverse range of antiviral and inflammatory genes compared to treatment with IFNα, and bioinformatics analysis identified distinct nodes of IRF7, IRF1 and NF-kB gene activation. Pre-treatment of A549 cells with 5’pppRNA dramatically blocked H1N1 A/PR/8/34 Influenza virus replication; furthermore, intravenous delivery of 5’pppRNA to BALB/c mice generated an antiviral response in mouse lungs that protected animals from a lethal challenge with H1N1 A/PR/8/34. RNA agonist delivery inhibited virus replication in mouse lungs within the first 24h after H1N1 challenge and protected the lungs of infected animals from virus-induced pathology. Finally, 5’pppRNA pre-treatment also completely or partially blocked replication of Dengue, Vaccinia and HIV-1 replication. These results illustrate that naturally derived RIG-I agonists represent a potent stimulator of the innate antiviral response, with the capacity to block replication of multiple pathogenic human viruses.

Termination of IFN signalling is likewise crucial to the proper maintenance of the innate and adaptive immune response to virus infection. We also identified an essential role for LUBAC-mediated linear ubiquitination of NEMO in the negative regulation of the RIG-I antiviral pathway through sequestration of TRAF3 from the MAVS adapter. LUBAC and NEMO-Ub constructs inhibited RIG-I signaling downstream of MAVS and upstream of TBK1; linearly ubiquitinated NEMO interacted physically with TRAF3, and disrupted the MAVS-TRAF3 complex, thus providing a mechanistic explanation for the downregulation of RIG-I signaling. Using SHARPIN deficient cpdm MEFs, we observed on the one hand, an increased and prolonged antiviral response, while on the other hand, an impaired NF-kB activation, indicating that linear ubiquitination is required for NF-kB activation downstream of RIG-I. Interestingly, an increase in apoptotic cell death was also detected in SHARPIN-deficient cpdm MEFs after VSV infection, potentially attributable to the absence of the anti-apoptotic activity of NF-kB. These studies reveal a novel negative feedback mechanism used by host cells to regulate the IFN antiviral response.

Disclosure of Interest: None Declared
Introduction: Type I interferons (IFNs) activate the JAK/STAT pathway and stimulate transcription from many IFN stimulated genes (ISGs). This process likely involves destabilization and reorganization of chromatin. However, chromatin events associated with ISG induction is poorly understood. To gain insight into a link between transcription and chromatin regulation, we studied whether IFN stimulation causes exchange of histones relevant to epigenetic regulation.

Methods: Because the histone H3.3 is implicated in transcription coupled chromatin change, we constructed NIH3T3 cells expressing GFP- H3.3 and examined H3.3 incorporation into ISGs by ChIP in parallel with ISG transcription and transcription factor recruitment.

Results: IFN stimulation led to rapid recruitment of RNA polymerase II and BRD4, an acetyl-histone binding factor to the ISG. This was followed by recruitment of the elongation factor P-TEFb, the pausing complex NELF/DSIF and SPT6. Along with these events, IFN stimulation caused rapid H3.3 accumulation in the ISGs. H3.3 accumulation was greater in the coding region and the gene end than in the promoter region where virtually no H3.3 incorporation was detected. Analysis with a BRD4 specific inhibitor JQ1 showed that H3.3 incorporation depended on BRD4 recruitment and ISG elongation. However, H3.3 incorporation into ISGs continued past ISG elongation, leaving the H3.3 mark on ISGs for at least two cycles of cell division. The mutant GFP-H3.3K36R was not incorporated into ISGs, indicating that methylation of K36 is required for H3.3 incorporation. Finally, H3.3 incorporation was also observed in another activation model, indicating the generality of transcription-induced histone exchange.

Conclusion: We show that IFN stimulation triggers rapid and extensive H3.3 incorporation into ISGs, which is presumably associated with expulsion of the preexisting H3 (H3.1/H3.2). This event required active ISG elongation, suggesting that passage of RNA polymerase II through the ISG gene body destabilizes the architecture of the RNA-DNA-nucleosome, necessitating reconstruction of nucleosomes composed of H3.3. Based on the remarkable persistence of the H3.3 mark left on the ISGs long after transcription, we suggest that transcription-induced H3.3 deposition represents an epigenetic mark linked to transcriptional memory.


Disclosure of Interest: None Declared

Presentation: EVASION OF THE OAS-RNASE L PATHWAY BY MURINE CORONAVIRUS NS2 PROTEIN IS REQUIRED FOR VIRAL REPLICATION AND HEPATITIS

Thursday, September 13, 2012 / 17:45 - 18:00

Abstract presenter: Babal K. Jha

Abstract: CYT012-1046: EVASION OF THE OAS-RNASE L PATHWAY BY MURINE CORONAVIRUS NS2 PROTEIN IS REQUIRED FOR VIRAL REPLICATION AND HEPATITIS

B. K. Jha 1; L. Zhao 2; A. Wu 2; R. Elliot 2; J. Ziebuhr 3; A. E. Gorbalenya 4; S. R. Weiss 2; R. H. Silverman 1

Cleveland Clinic Lerner Research Institute, Cleveland, University of Pennsylvania, Philadelphia, United States; Justus Liebig University Giessen, Giessen, Germany; Leiden University Medical Center, Leiden, Netherlands

Introduction: The 2',5'-oligoadenylate synthetase-ribonuclease L (OAS-RNase L) system is a potent IFN induced antiviral pathway. Following infection, IFNs induce a group of OAS genes whose products
are activated by viral double-stranded RNA. OAS uses ATP to generate 2',5'-linked oligoadenylates (2-5A). 2-5A binds to and activates the ubiquitous cellular endoribonuclease RNase L causing cleavages of single stranded regions of both viral and cellular RNA thus inhibiting viral replication. In addition, detection of the newly generated short RNAs by cellular pattern recognition receptors, MDA5 and RIG-I, further enhances IFN production and the ensuing antiviral activities. The intracellular concentration of 2-5A is believed to be the primary factor controlling RNase L activation. The liver contains abundant innate immune cells, which provide the first line of defense against pathogens. However, the factors that determine whether a virus can bypass this defense to access and infect the liver parenchyma are not well understood. The murine coronavirus, mouse hepatitis virus (MHV), strain A59, infection of mice provides a model for virus induced hepatitis. The MHV accessory protein, ns2, antagonizes the type I IFN response in macrophages and promotes the induction of hepatitis. Here we will describe how the ns2 protein facilitates the development of viral hepatitis by blocking OAS-RNase L pathway.

**Methods:** Bone marrow macrophages (BMM) from wild type (wt) and RNase L-/- mice were infected with A59 and ns2 mutant MHV. Viral titers were determined by plaque assays. RNase L activity was monitored by rRNA integrity in RNA chips. Intracellular levels of 2-5A were measured using RNase L activation assays. Effects of ns2 on 2-5A levels in cells were determined by transfecting ns2 or mutant ns2 cDNAs into HEK-293T cells. Recombinant ns2 and mutant ns2H126R proteins were purified and incubated with 2-5A in vitro and the 2-5A breakdown products were measured by HPLC. Hepatitis was determined by histology following inoculation A59 or ns2 mutant MHV into wt and RNase L-/- mice.

**Results:** We found evidence for a new molecular mechanism of subversion of the RNase L pathway in macrophages that regulates acute hepatitis during MHV infection. Coronavirus ns2 belongs to the LigT-like protein family, within the 2H phosphoesterase superfamily, some of which possess cyclophosphodiesterase activity(CPD). We have found that ns2 is not a CPD, but instead is a 2',5'- phosphodiesterase (PDE) that cleaves, and thus eliminates 2-5A, the activator of RNase L. We observed that ns2 blocks the IFN inducible OAS-RNase L pathway to facilitate hepatitis development. Ns2 prevents activation of RNase L and consequently limits viral RNA degradation. An ns2 mutant virus was unable to replicate in the liver or induce hepatitis in wt mice, but was highly pathogenic in RNase L-/- mice. Thus, RNase L is a critical cellular factor for protection against viral infection of the liver and the resulting hepatitis.

**Conclusion:** MHV accessory protein ns2 is a 2',5'-PDE which degrades 2-5A and limits RNase L activation thus facilitating virus-induced hepatitis in mice.

**Disclosure of Interest:** B. Jha: None Declared, L. Zhao: None Declared, A. Wu: None Declared, R. Elliot: None Declared, J. Ziebuhr: None Declared, A. Gorbalenya: None Declared, S. Weiss: None Declared, R. Silverman Consultant for: Alios Biopharma

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**Presentation:** TLR3 AND RIG-I SENSING OF HCV INFECTION BY HEPATOCYTES LEADS TO INTERFERON-INDEPENDENT CXCL10 INDUCTION

*Thursday, September 13, 2012 / 18:00 - 18:15*

**Abstract presenter:** Jessica Brownell

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**Abstract:**

**CYTO12-1136: TLR3 AND RIG-I SENSING OF HCV INFECTION BY HEPATOCYTES LEADS TO INTERFERON-INDEPENDENT CXCL10 INDUCTION**

J. Brownell 1,*, J. Wagoner 2, D. Thirstrup 2, W. Smith 3, K. Li 4, S. Polyak 2

University of Washington, Seattle, University of Tennessee Memphis, Memphis, United States

**Introduction:** Chronic hepatitis C is characterized by a persistent hepatic inflammatory response and the recruitment of immune effector cells to the liver by pro-inflammatory chemokines. The chemokine CXCL10 is induced by HCV infection *in vitro* and *in vivo*, and is correlated with the outcome of Interferon (IFN)-based therapies. Therefore, we investigated how sensing of HCV infection by the pathogen recognition receptors (PRRs) TLR3 and Retinoic Acid Inducible Gene 1 (RIG-I) led to expression of CXCL10 in hepatocytes.
Methods: Primary human hepatocytes were infected in vitro with the HCV clone JFH-1. CXCL10 production was measured via real-time RT-PCR, Luminex Bead Array, and immunofluorescence. Type I and type III interferon induction was measured directly by real-time PCR and indirectly by IFIT1 (ISG56) activation. CXCL10 production was also measured in response to RIG-I-specific (Sendai Virus) and TLR3-specific (poly:C) stimuli as well as siRNA knockdown of RIG-I and TLR3 in PH5CH8 hepatocyte cultures. Transcription factors involved in CXCL10 induction were identified using CXCL10 promoter-Luciferase reporter constructs. Experiments were also performed in Huh7 hepatoma-derived cell lines that expressed either one, both, or neither PRR.

Results: CXCL10 mRNA and protein expression were detected following HCV infection of both human hepatoma cell lines and primary human hepatocytes. siRNA knockdown of TLR3 and RIG-I protein abrogated CXCL10 induction in response to PRR-specific agonists. Huh7-derived cells expressing both TLR3 and RIG-I produced maximal CXCL10 mRNA with negligible induction of type I or III IFN. Furthermore, neutralization of type I and type III IFN did not impact CXCL10 induction during HCV infection while IFIT1 activation was completely abolished. Immunoﬂuorescence studies revealed a direct positive correlation between intracellular HCV core and CXCL10 protein expression. Finally, TLR3 and RIG-I-specific agonists activated CXCL10 transcription in a NF-κB-dependent manner.

Conclusion: Collectively, the data suggest that CXCL10 induction during HCV infection derives directly from PRR signaling to NF-κB activation and is marginally influenced by paracrine/autocrine IFNs. Direct induction via both TLR3 and RIG-I pathways may indicate the existence of uncharacterized crosstalk within the canonical innate immune signaling cascade and may reveal host-directed drug targets for anti-inflammatory therapies.

Disclosure of Interest: None Declared
MDA5 and CD4 T cells. Moreover, we found that chronic LCMV infection rapidly attenuated the IFN-I response while early administration of exogenous IFN-I rescued the CD8 T cell response promoting viral clearance.

**Conclusion:** We conclude that induction of effective antiviral CD8 T cells depends on the timing and magnitude of the IFN-I response. IFN-I production by pDCs is too little and too transient to facilitate CD8 T cell responses to LCMV. The magnitude and duration of MDA5-induced IFN-I is adequate to elicit effective CD8 T cell responses and control infection during acute, but not chronic infection, which replicates more rapidly and impairs both MDA5 and CD4 T cell function. Thus, timely administration of exogenous IFN-I may rescue CD8 T cell functions during human chronic viral infections.

**Disclosure of Interest:** None Declared

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**Parallel session - Cytokines in Inflammation and metabolism**

*Friday, September 14, 2012 / 14:00 - 16:00*

**Room:** Room 3&4  
**Type:** Parallel session  
**Chairperson:** Manfred Kopf (Switzerland)

**Presentation: Intracellular metabolic pathways control immune tolerance**  
*Friday, September 14, 2012 / 14:00 - 14:30*

**Invited speaker:** Giuseppe Matarese (Italy)

**Abstract:**

**CYTO12-1334: INTRACELLULAR METABOLIC PATHWAYS CONTROL IMMUNE TOLERANCE**  
G. Matarese 1,∗  
SALERNO UNIVERSITY, Salerno, Italy

Abstract: The field that links immunity and metabolism is rapidly expanding. Apparently non-immunological disorders such as obesity and type 2 diabetes have been linked to immune dysregulation suggesting that metabolic alterations can be induced by or be consequence of an altered self-immune tolerance. In this context, a key role is played by signalling systems acting as metabolic “sensors” linking energy/nutritional status to regulatory T (Treg) cell functions such as the adipose tissue derived hormone, leptin. We propose that a dynamic/oscillatory activity of intracellular metabolism might represent a shift in understanding the molecular mechanisms governing Treg cell tolerance. In particular, the decision between Treg cell proliferation and hyporesponsiveness arises from their ability to probe the extracellular milieu and, modulating the metabolic intracellular signalling, to determine different qualitative and quantitative functional outcomes.

**Disclosure of Interest:** None Declared

**Presentation: Inflammasome activation in chronic inflammatory diseases**  
*Friday, September 14, 2012 / 14:30 - 15:00*

**Invited speaker:** Eicke Latz (Germany)

**Presentation: PREDOMINANT ROLE OF MACROPHAGE-DERIVED IL-1ALPHA BUT NOT IL-1BETA IN ATHEROSCLEROSIS**  
*Friday, September 14, 2012 / 15:00 - 15:15*
Abstract: CYTO12-1234: PREDOMINANT ROLE OF MACROPHAGE-DERIVED IL-1ALPHA BUT NOT IL-1BETA IN ATHEROSCLEROSIS
S. Freigang 1, F. Ampenberger 1, M. Hersberger 2, M. Kopf 1
ETH Zurich, University Children's Hospital Zurich, Zurich, Switzerland

Introduction: Atherosclerosis is a chronic inflammatory condition affecting the arterial vasculature. Its clinical manifestations, such as myocardial infarction and ischemic stroke, are the leading causes of morbidity and mortality worldwide. IL-1 receptor (IL-1R)-signaling severely exacerbates the chronic vascular inflammation that drives disease progression. While the contribution of inflammasome-activation to atherogenesis in vivo remains controversial, IL-1beta is currently targeted in clinical trials. However, as both IL-1alpha and IL-1beta signal via IL-1R and exhibit overlapping functions, the relative contribution of each IL-1 isoform to atherogenesis is still unclear.

Methods: We have compared the impact of both IL-1 isoforms on disease progression in mouse models of atherosclerosis, and have investigated the mechanisms of IL-1alpha versus IL-1beta induction by different atherogenic lipids in macrophage-foam cells.

Results: Here we report that deficiency in hematopoietic IL-1alpha markedly reduced atherosclerosis by 51%, whereas no significant effect was observed in mice lacking BM-derived IL-1beta, suggesting that macrophage-derived IL-1alpha - and not IL-1beta - represents the pro-atherogenic IL-1 isoform. Moreover, our study identified the fatty acid oleic acid, which is abundant amongst plaque lipids, as a selective trigger of potent IL-1alpha - but not IL-1beta - responses in foam cells. This atherogenic IL-1alpha production was independent of inflammasome activation, but surprisingly required the cellular expression of pro-IL-1beta. Hence, IL-1beta/- macrophages were unable to secrete IL-1alpha, which may explain the conflicting reports showing reduced atherosclerosis in IL-1beta-deficient mice. Furthermore, feeding of an oleate-enriched but cholesterol-free diet aggravated atherosclerosis, suggesting that IL-1alpha is both necessary and sufficient to induce the development of atherosclerotic plaques.

Conclusion: Taken together, our data demonstrate that macrophage-foam cells promote atherogenesis primarily through production of IL-1alpha, and therefore suggest that the role of inflammasome activation and IL-1beta in atherosclerosis may need to be reconsidered. Most importantly, they indicate that IL-1alpha and not IL-1beta should be targeted therapeutically in patients with cardiovascular disease.

Disclosure of Interest: None Declared

Presentation: PROLONGED EXPOSURE TO TYPE-I IFN INHIBITS IL-10 SIGNALING IN MEMORY AND REGULATORY T CELLS: A NEW MECHANISM CONTRIBUTING TO DEVELOPMENT OF AUTOIMMUNE DISEASES

Friday, September 14, 2012 / 15:15 - 15:30

Abstract presenter: Giorgio Raimondi

Abstract: CYTO12-1078: PROLONGED EXPOSURE TO TYPE-I IFN INHIBITS IL-10 SIGNALING IN MEMORY AND REGULATORY T CELLS: A NEW MECHANISM CONTRIBUTING TO DEVELOPMENT OF AUTOIMMUNE DISEASES
Y. Nicholson 1, L. Chen 1, G. Raimondi 1, 1
1Starzl Transplantation Institute - University of Pittsburgh School of Medicine, Pittsburgh, United States

Introduction: Interleukin 10 (IL-10) is an immunoregulatory cytokine necessary to prevent autoimmune disease development. In addition to its inhibitory influence on antigen-presenting cells, IL-10 has been shown recently to act also through modulation of effector T cell and memory T cell (Tmem) function and optimization of regulatory T cell (Treg) suppressive activity. Mice with Tmem- or
Abstract presenter: Fabrizio Montecucco

CYTO12-1107: TREATMENT WITH CHEMOKINE-BINDING PROTEIN EVASIN-3 REDUCES ATHEROSCLEROTIC VULNERABILITY FOR ISCHEMIC STROKE IN MICE

F. Montecucco 1, R. da Silva 2, R. A. Fraga-Silva 3, L. Capettini 2, S. Quintao 2, S. Lenglet 1, G. Pelli 1, K. Galan 1, V. Braunersreuther 1, K. Schaller 1, A. E. Proudfoot 4, N. Stergiopulos 3, R. A. Santos 2, Y. Gasche 5, F. Mach 1, J.-C. Copin 1

1University of Geneva, 2Merck-Serono, Geneva, 3EPFL, Lausanne, Switzerland, 4University of Minas Gerais, 5Geneva University Hospital, Belo Horizonte, 2University of Minas Gerais, 5Geneva University Hospital, Geneva, Brazil

Introduction: The presence and rupture of a carotid plaque inducing a severe arterial lumen stenosis have been associated with a consequent acute ischemic stroke in the brain. Treatment with chemokine-binding proteins isolated from tick salivary glands (also called “Evasins”) has been shown...
to reduce leukocyte recruitment in animal models of several diseases, such as acute myocardial infarction and rheumatoid arthritis. In the present study, we investigated the potential benefits of the selective inhibition of neutrophil recruitment (mediated by Evasin-3 treatment) in both primary and secondary prevention of ischemic stroke.

Methods: Two mouse models of shear stress-induced carotid atherosclerosis and cerebral ischemia and reperfusion were used (for ischemic stroke primary and secondary prevention strategies, respectively). In the first model, preventive chronic treatment with Evasin-3 (5 daily injections [at 1 microg/mouse] per week for total 3-week treatment period) was tested as compared with control vehicle (PBS) in ApoE-/- mice submitted to the implantation of “cast” carotid device. In the second model, C57Bl/6 mice aged from 8 to 12 weeks were used. The left middle cerebral artery (MCA) was occluded for 45 min with an intraluminal Dafilon 6.0 nylon suture. Then, blood flow was re-established and mice were followed up for 24h of reperfusion. Five minutes after MCA occlusion, mice received one intraperitoneal injection of Evasin-3 (10 microg/mouse) or control vehicle (PBS). Leukocyte infiltration, infarct size and blood brain barrier (BBB) permeability were determined at 24h of reperfusion.

Results: In atherosclerotic plaques of ApoE-/- mice, primary prevention treatment with Evasin-3 was associated with the reduction of neutrophils and MMP-9 content in aortic root and carotid plaques as compared to control vehicle. Secondary prevention treatment approach (after ischemic stroke) with Evasin-3 was associated with a significant reduction in neutrophil infiltration in the ischemic brain as compared to control vehicle. However, this anti-inflammatory effect was not associated with any improvements in cerebral infarct size, edema and BBB permeability.

Conclusion: Primary prevention strategy of Evasin-3 treatment reduced atherosclerotic plaque vulnerability for ischemic stroke. Acute Evasin-3 administration after cerebral ischemia onset significantly reduced brain inflammation, but failed to improve post-stroke outcomes.

References: None.

Disclosure of Interest: None Declared

Presentation: GLUCOCORTICOID RECEPTOR DIMERIZATION INDUCES MKP1 TO PROTECT AGAINST TNF-INDUCED INFLAMMATION

Friday, September 14, 2012 / 15:45 - 16:00

Abstract presenter: Sofie Vandevyver

Abstract:

CYTO12-1111: GLUCOCORTICOID RECEPTOR DIMERIZATION INDUCES MKP1 TO PROTECT AGAINST TNF-INDUCED INFLAMMATION

S. Vandevyver 1,2, L. Dejager 1, T. Van Bogaert 1, A. Kleyman 2, Y. Liu 3, J. Tuckermann 4, C. Libert 1
1DMBR/VIB, Zwijnaarde, Belgium, 2Leibniz Institute for Age Research, Jena, 4Institute for General Zoology and Endocrinology, Ulm, Germany, 3Department of Pediatrics, Ohio State University, College of Medicine, Ohio, United States

Introduction: Glucocorticoids acting through the glucocorticoid receptor (GR) inhibit TNF-induced lethal inflammation. We demonstrate the increased TNF sensitivity of mice expressing a mutant GR (GRdim/dim), which does not dimerize or induce GRE genes. Induction of MKP-1 seems essential for GR control of TNF-induced inflammation: TNF fails to induce the MKP-1-encoding gene Duspl in GRdim/dim mice, and MKP-1-/- mice are strongly sensitized to TNF. This sensitization was shown by increased inflammatory gene induction in livers, circulating cytokines, cell death in intestinal epithelium, and by severe intestinal inflammation, hypothermia and death. Because JNK phosphorylation is significantly higher in livers of MKP-1-/- mice, JNK-deficient mice were studied for their response to TNF. Although JNK-1/- mice showed no change in sensitivity to TNF, JNK-2/- mice were
significantly protected against TNF, which identifies JNK-2 as an essential player in inflammation induced in vivo by TNF. Using MKP-1/JNK-2 and GR\textsuperscript{dim/dim}/JNK-2 double deficient mice, which are significantly less sensitive to TNF than MKP-1\textsuperscript{-/-} and GR\textsuperscript{dim/dim} mice, respectively, we provide evidence that the increased sensitivity of MKP-1\textsuperscript{-/-} and GR\textsuperscript{dim/dim} mice to TNF is partly due JNK-2. Our data show that dimerization of GR critically induces MKP-1 and hence controls JNK-2, an essential mediator of TNF-induced apoptosis and lethal inflammation.

Methods:
Results:
Conclusion:
References:
Disclosure of Interest: None Declared