“The biology and clinical application of Lymphoid Stress-Surveillance”

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Immunity
Perspective

γδ T Cells and the Lymphoid Stress-Surveillance Response

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Important note -

For the benefit of the most effective dissemination of knowledge and its discussion, this presentation includes unpublished data relating to the effect of lymphoid stress-surveillance on immunoglobulin production, and on the effects of gamma delta T cell re-activation in patients with advanced breast cancer. These data cannot be cited or reproduced for any purpose without the authors’ written and specific permission.
Immunology and body surfaces

Epithelial tissues are sites of entry and replication of myriad pathogens, including HIV. Major inflammatory disorders such as IBD and psoriasis can also occur. Langerhans cells are involved in immune responses at these surfaces.
Conventional lymphocyte surveillance

microbes

TLR

DC

TISSUE

Lymph node selection
Immune surveillance of epithelial tissues - gap 1
- The epithelial cell itself
gap 2-
Non microbial stress
Immune surveillance of stressed tissues

“Stress-beacon”
C-type Lectin
ATP
urate

stress

?
Lymphoid stress-surveillance

“Stress-Antigens”

Epithelial cells
Tissue-resident T cells

High responder frequency
- No lymph node selection

gap 3- “unconventional T Cells & Intraepithelial Lymphocytes
15µm
Lymphoid stress-surveillance: evidence for high frequency responses

T10/T22  Peptide-MHC
1 in 200  1 in 10^6

Dendritic Epidermal T cells
(DETC)  Vγ5  Vδ1
High Frequency Response of Human $\gamma\delta$ cells to common microbial metabolite or “self stress signal”

**Mevalonate pathway (host)**

- Acetyl CoA
  - Acetoacetyl CoA
  - HMG-CoA
  - Mevalonate
  - Mevalonate-PP

**MEP pathway (Eubacteria; protozoa)**

- Pyruvate + GADPH
  - DOXP
  - MEP
  - HMB-PP 1pM

Isopentenyl pyrophosphate (IPP) 10nM

Geranyl pyrophosphate

Farnesyl pyrophosphate synthase

Farnesyl pyrophosphate

protein geranyl-geranylation, protein farnesylation, cholesterol, ubiquinone, dolichol,
Lymphoid stress-surveillance: another route to obtaining high frequency responses

**Diagram:**
- T cells (red) and MHCII+ LC (blue) are shown.
- MICA/Rae-1=NKG2D interaction is highlighted.
- DETC and NKG2D / control are indicated on the graph.

**Text:**
- MICA/Rae-1=NKG2D interaction.
- DETC and NKG2D / control on the graph.
MHC versus NKG2D ligands

Many NKG2D ligands are stress-regulated e.g. genotoxic shock

Many are expressed by tumours

Tumours (and viruses) often display immune evasion strategies
Is NKG2D-ligand upregulation on normal cells an immune activator?
Jessica Strid (Nature Immunology, 2008)

Transgenic mice induced to express Rae-1:

- **Line 166**
  - Rae-1 under control of tetracycline-dependent promoter

- **Line 349**
  - Tetracycline-dependent transcriptional activator under involucrin promoter

- **Inv-Rae** 'bitransgenic'

- **DOX**

- **Induced epidermal expression of Rae-1**
Effects of Inducing Rae-1β

Inv-rtTA siTg  Inv-Rae-1 biTg

**γδ TCR (GL3)**  **MHCII (2G9)**

DETC (NKG2D⁺) round-up
(DAP10→vav
upregulate CD69
downregulate TC

LC (NKG2D⁻) round-up;
upregulate CD86 etc.

Conclude: resident tissue-associated immune compartments are highly
dynamic in response to altered expression of self "stress-antigen"
Effects of Inducing Rae-1β

Confocal microscopy of epidermal sheets (120h)

Conspicuous αβ T cell infiltration
72 post-induction (founder 16)

Single tg

MHCII
TCRαβ

bi-tg
The scope of lymphoid stress-surveillance

A

infection

TLR

B

stress

Stress-ligand for NKG2D

TCR / NKG2D

?
OVA skin-patch experiment following induction of Rae-1 - primary response to OVA

Jessica Strid; Olga Sobolev - unpublished

Experimental time-line:

0 6 7 16

Given DOX diet (Rae-1 induced in BiTg mice)  Back/neck skin shaved with surgical blade  Patch with 100mg OVA or PBS applied to shaved skin  Blood, spleen, LNs and skin collected

Adjuvant activity of acute stress – Tumour immunogenicity

[Graph showing OVA-specific LN proliferation]
The value of the $\gamma\delta$ cell stress-surveillance system

Squamous cell carcinoma

DMBA + TPA treatment of immunological mutant mice
e.g. TCR$\delta^-$ and $\gamma5\delta1^-$ "double knockout" mice

Mouse

Human
ed susceptibility to inflammatory chemical carcinogenicity of mice lacking $V\gamma 5V\delta 1^+$ DETC
1+ DETC act early, consistent with stress-surveillance

<table>
<thead>
<tr>
<th>Mouse Strain</th>
<th>Tumors / Mouse</th>
<th>P-value 1</th>
<th>Carcinomas / Mouse</th>
<th>P-value 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>TCRδ−/−</td>
<td>11.08 ± 1.74</td>
<td>≤0.002</td>
<td>5.66 ± 1.24</td>
<td>≤0.05</td>
</tr>
<tr>
<td>Vγ5−/−</td>
<td>5.83 ± 1.09</td>
<td>N.S.</td>
<td>3.58 ± 0.99</td>
<td>N.S.</td>
</tr>
<tr>
<td>Vδ1−/−</td>
<td>6.07 ± 0.95</td>
<td>N.S.</td>
<td>3.79 ± 0.73</td>
<td>N.S.</td>
</tr>
<tr>
<td>Vγ5−/−Vδ1−/−</td>
<td>11.17 ± 1.36</td>
<td>≤0.0004</td>
<td>7.08 ± 1.05</td>
<td>≤0.01</td>
</tr>
<tr>
<td>WT</td>
<td>5.08 ± 0.84</td>
<td>-</td>
<td>3.38 ± 0.75</td>
<td>-</td>
</tr>
</tbody>
</table>

1 Mean values at week 17 post-DMBA initiation.

2 P-values versus WT; N.S., not significant.

Progression ratio

w.t .665
sko .614
dko .634
Lymphoid Stress Surveillance

High response frequencies ✓

Scope ✓

Problems
There maybe a limited capacity to eradicate chronic infections, related to which is our struggle to make vaccines against them.
Surprising partners in chronic infections

Parasitic worms and IL10
Does chronic stress-surveillance immune suppression

- **Given DOX diet (Rae-1 induced in BiTg mice)**
- **Day 0**: Back/neck skin shaved with surgical blade
- **Day 270**: Patch with 100mg OVA or PBS applied to shaved skin
- **Day 271**: Blood, spleen, LNs and skin collected
- **Day 280**:
Chronic "stress-surveillance" reverts to an immuno-suppressive state

OVA-specific proliferation:

Acute Rae-1 (5 days)

Long-term Rae-1 (9 months)

OVA-specific LN proliferation

OVA-specific LN proliferation

OVA-specific immunoglobulins

OVA-specific immunoglobulins
"stress-surveillance" reverts to an immuno-suppres
Rejuvenating Stress-Surveillance
**Rejuvenating T cells Responses in the Clinic**

Mevalonate pathway (host)

- **Mevastatin**

  - Acetyl CoA
  - Acetoacetyl CoA
  - HMG-CoA
  - Mevalonate
  - Mevalonate-PP

  \[ \text{Mevastatin} \]

MEP pathway (Eubacteria; protozoa)

- Pyruvate + GADPH
  - DOXP
  - MEP
  - HMB-PP 1pM

  \[ \text{MEP pathway (Eubacteria; protozoa)} \]

**Isopentenyl pyrophosphate (IPP) 10nM**

- Geranyl pyrophosphate
- Farnesyl pyrophosphate
  - protein geranyl-geranylation, protein farnesylation, cholesterol, ubiquinone, dolichol,

**Aminobisphosphonates (zoledronate)**

- **Isopentenyl pyrophosphate (IPP) 10nM**
Clinical Trial
Francesco Dieli - Palermo

Targeting Human γδ T Cells with Zoledronate and Interleukin-2 for Immunotherapy of Hormone-Refractory Prostate Cancer

Francesco Dieli,1 David Vermijlen,3 Fabio Fulfaro,2 Nadia Caccamo,1 Serena Meraviglia,1 Giuseppe Cicero,2 Andrew Roberts,3 Simona Buccheri,1 Matilde D’Asaro,1 Nicola Gubbia,2 Alfredo Salerno,1 Matthias Eberl,1,5 and Adrian C. Hayday3

administration of zoledronate (1 mg i.v.) - low m.w. activator of Vγ9+ T cells; safe; used in metastasis

+/- a low dose of IL-2 (0.6 million U IL-2 s.c.) (Francesco Dieli, University of Palermo) - safe;

effects on γδ cells are the only ones


$\gamma\delta$ cell subsets

$T_{naive} \text{CD}45\text{RA}^+\text{CD}27^+$

$T_{CM} \text{CD}45\text{RA}^-\text{CD}27^+$

$T_{EM} \text{CD}45\text{RA}^-\text{CD}27^-$

$T_{EMRA} \text{CD}45\text{RA}^+\text{CD}27^-$
γδ cell responses

<table>
<thead>
<tr>
<th>Months after treatment</th>
<th>0</th>
<th>3</th>
<th>9</th>
<th>12</th>
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<tbody>
<tr>
<td>CSFE</td>
<td>IFN-γ</td>
<td>IFN-γ</td>
<td>IFN-γ</td>
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</tr>
</tbody>
</table>
γδ cell responses

- **Proliferation**
  - Stimulation Index ± SD
  - Months: 0, 3, 9, 12

- **IFN-γ**
  - ng/ml ± SD
  - Months: 0, 3, 9, 12

- **BLT-esterase**
  - O.D. 405 nm ± SD
  - Months: 0, 3, 9, 12
Figure 8

(A) Total γδ cells/µl

(B) T_EM γδ cells/µl

(C) Total γδ cells/µl

(D) T_EM γδ cells/µl

clinical outcome
Correlations of T cell activity and outcome
Data so far......TRAIL vs outcome......
In vivo manipulation of Vγ9Vδ2 T cells with zoledronate and low dose interleukin-2 for immunotherapy of advanced breast cancer patients.

Serena Meraviglia¹, Matthias Eberl², David Vermijlen³, Matilde Todaro⁴, Simona Buccheri⁵, Giuseppe Cicero⁴, Carmela La Mendola¹, Giuliana Guggino¹, Matilde D’Asaro¹, Valentina Orlando¹, Francesco Scarpa¹, Andrew Roberts⁶, Nadia Caccamo¹, Francesco Dieli¹*, Giorgio Stassi⁴, and Adrian C. Hayday⁶*.
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