Cytokines in Malignancy

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iSBTc 11/1/07
Immunobiology of Cytokines

For discussion
- Cytokine structure
- Stimuli leading to cytokine synthesis
- Cell(s) responsible for cytokine production
- Cytokine-responsive cell(s)/receptor structure
- Signaling induced by cytokine binding
- Preclinical/clinical status of cytokine

Not for discussion
- Cytokines in the pathogenesis of malignancy
- Other clinical aspects of cytokine manipulation
  - Autoimmune disease
  - Infectious disease
- Homeostatic cytokines
Cytokines during iSBTc 2007

- IL-2, many sessions, especially reconstitution and cellular therapies
- IL-7, Crystal Mackall, Friday Nov 2, p.m.
- IL-12, Mehmet Kilinc, Friday Nov 2, p.m.
- IL-24, Nancy Poindexter, Fri Nov 2, p.m.
T cells are mobilized when they encounter a cell such as a macrophage or a B cell that has digested an antigen and is displaying antigen fragments bound to its marker molecules.

The T cell, alerted and activated, secretes lymphokines. Some lymphokines spur the growth of more T cells. Some T cells become killer cells and track down body cells infected by viruses.

Some lymphokines attract immune cells — fresh macrophages, granulocytes, and other lymphocytes — to the site of infection. Yet other lymphokines direct the recruits once they arrive on the scene.

Lymphokines help the T cell to mature.
Induction of an immune response

Recognition       Processing       Presentation       Activation

Erkennung         Prozessierung    Präsentation       Aktivierung

Antigen → APZ → B7-1 (CD80) → CD28 → CD4 → T_H
MHC II → Peptid → TCR

IL-2R → IL-2
Cytokine and Cell Interactions

- IL-10
- GM-CSF
- IL-2
- IL-7
- IL-15
- IL-12(+)
- IL-18
- IL-21
- IL-6
- IL-10(-)
- IL-4, IL-13
- IL-10
- IMMUNE SYSTEM 101-201 for Primer Attendees

- IL-10
- GM-CSF
- APC
- CCR5
- IL-1
- TNF-α
- Release of tumour-specific antigen
- Phagocytosis
- Immature APC
- Maturation
- Activated mature APC
- MHC Cl II
- CD40
- CCR7
- ELC
- Endothelial cell in lymph node
- Th
- Migration
- Activation of APC
- Co-stimulation
- CTL
- Proliferation
- Co-stimulation

APCs (including macrophages and dendritic cells) are recruited into the tumour site by MIP-1α.
Generic cytokine signaling
GM-CSF as immunotherapy

• Cells of origin
  – Th1, Th2
  – Others include epithelial, fibroblast, *tumor*
• Target cell: immature DC (& myeloid progenitor)
• Biological functions
  – Stimulation of T cell immunity via effect on APC
  – Myeloid cell proliferation, differentiation
• Clinical development
  – Hematopoietic support
  – Not a potent stand-alone cytokine in cancer
  – Transgenic expression (GVAX)
  – Adjunct to immunotherapy
    • Uncontrolled data for benefit in adjuvant Rx of melanoma
    • Phase III results pending +/- peptide
GM-CSF Xgenic Tumor “GVAX”
Interferons

- **Type I**
  - Alpha interferons: produced by WBC, mφ
  - Beta interferon: produced by fibroblasts, epithelial cells

- **Type II**
  - IFN-γ: produced by T and NK cells
  - Extensive range of targets

- **Immunomodulatory effects**
  - MHC class I/II upregulation
  - Modulation of T/NK cell cytolytic activity
  - Modulation of macrophage/DC function

- **Direct effects on tumor cells**
  - MHC upregulation
  - Antiproliferative/pro-apoptotic effects

- **Anti-angiogenic effects**
  - IP-10
  - Thrombospondin
IFN-α Signaling
IFN-γ Signaling

- IFN-γ binding to γR1 and γR2
- Recruitment and Phosphorylation of Stat1α
- Dimerization and Nuclear Translocation
Rates of Recurrent Melanoma: High-Dose and Low-Dose IFN

- HDI reduced risk of disease recurrence by 26%, $P_2 = 0.00009$
- Trend for increased benefit with high dose, $P = 0.02$

**High Dose Trials**
- ECOG 1684
- Intergroup E1690 (H)
- NCCTG 83-7052
- ECOG 2696
- Subtotal:

**Low Dose Trials**
- WHO 16
- Intergroup E1690 (L)
- UKCCCR AIM-High
- French CGM
- Austrian MMCG
- Scottish MG
- Subtotal:
<table>
<thead>
<tr>
<th>Condition</th>
<th>Any Severity</th>
<th>Grade 3</th>
<th>Grade 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fatigue*</td>
<td>137 (96)</td>
<td>39 (27)</td>
<td>6 (4)</td>
</tr>
<tr>
<td>Neutropenia/Leukopenia</td>
<td>132 (92)</td>
<td>37 (26)</td>
<td>1 (1)</td>
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<tr>
<td>Fever*</td>
<td>116 (81)</td>
<td>26 (18)</td>
<td>0</td>
</tr>
<tr>
<td>Myalgia*</td>
<td>107 (75)</td>
<td>27 (19)</td>
<td>2 (1)</td>
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<tr>
<td>Anorexia*</td>
<td>99 (69)</td>
<td>14 (10)</td>
<td>2 (1)</td>
</tr>
<tr>
<td>Vomiting/Nausea</td>
<td>95 (66)</td>
<td>5 (3)</td>
<td>0</td>
</tr>
<tr>
<td>Increased SGOT</td>
<td>90 (63)</td>
<td>20 (14)*</td>
<td>0†</td>
</tr>
<tr>
<td>Headache*</td>
<td>89 (62)</td>
<td>25 (17)</td>
<td>1 (1)</td>
</tr>
<tr>
<td>Chills*</td>
<td>77 (54)</td>
<td>23 (16)</td>
<td>0</td>
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<tr>
<td>Depression</td>
<td>57 (40)</td>
<td>9 (6)</td>
<td>3 (2)</td>
</tr>
<tr>
<td>Diarrhea</td>
<td>50 (35)</td>
<td>0</td>
<td>0</td>
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<tr>
<td>Alopecia</td>
<td>42 (29)</td>
<td>2 (1)</td>
<td>0</td>
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<tr>
<td>Altered Taste Sensation</td>
<td>34 (24)</td>
<td>3 (2)</td>
<td>0</td>
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<tr>
<td>Dizziness/Vertigo*</td>
<td>33 (23)</td>
<td>3 (2)</td>
<td>0</td>
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<tr>
<td>Anemia</td>
<td>31 (22)</td>
<td>2 (1)</td>
<td>1 (1)</td>
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</table>

*Consistent with "flu-like illness."
†2/143 (1.4%)—grade 5.
<table>
<thead>
<tr>
<th>Autoantibodies or Manifestations of Autoimmunity</th>
<th>All Patients (N=200)</th>
<th>Induction-Therapy Group (N=96)</th>
<th>Extended-Therapy Group (N=104)</th>
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<tbody>
<tr>
<td>Autoantibodies or autoimmune disorders</td>
<td>52 (26)</td>
<td>23 (24)</td>
<td>29 (28)</td>
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<tr>
<td>Antithyroid antibodies</td>
<td>43 (22)</td>
<td>16 (17)</td>
<td>27 (26)</td>
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<tr>
<td>Antinuclear antibodies</td>
<td>12 (6)</td>
<td>2 (2)</td>
<td>10 (10)</td>
</tr>
<tr>
<td>Anticardiolipin antibodies</td>
<td>10 (5)</td>
<td>2 (2)</td>
<td>8 (8)</td>
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<tr>
<td>Vitiligo</td>
<td>11 (6)</td>
<td>5 (5)</td>
<td>6 (6)</td>
</tr>
<tr>
<td>Clinical manifestations</td>
<td>19 (10)</td>
<td>2 (2)</td>
<td>17 (16)</td>
</tr>
<tr>
<td>With autoantibodies</td>
<td>16 (8)</td>
<td>2 (2)</td>
<td>14 (13)</td>
</tr>
<tr>
<td>Without autoantibodies (vitiligo)</td>
<td>3 (2)</td>
<td>1 (1)</td>
<td>2 (2)</td>
</tr>
<tr>
<td>Multiple manifestations of autoimmunity</td>
<td>16 (8)</td>
<td>1 (1)</td>
<td>15 (14)</td>
</tr>
</tbody>
</table>

* Patients in the induction-therapy group received interferon alfa-2b (15 million IU per square meter of body-surface area per day, intravenously, five days per week for four weeks) followed by observation. Patients in the extended-therapy group received the same induction dose for 4 weeks, followed by subcutaneous therapy (10 million IU per day thrice weekly) for an additional 48 weeks.

Autoimmunity and benefit from adjuvant IFN-a2b
Interleukin-2

- Short chain type I cytokine
- Four $\alpha$-helical bundles
- Produced by activated T cells
- TCR/CD3 engagement plus CD28 ligation required
- Main targets are T, NK cells
- Stimulates immune responses and prevents tolerance
- Also downregulates immune response: role in $T_{reg}$ development/activity
Interleukin-2

- “T cell growth factor”
- Produced by Th1 cells for T cells but…
- Many other cells express IL-2R
  - B, NK/NKT, monocytes
  - Variable affinity depending on subunit expression
  - Response to IL-2 depends on cell type, receptor, milieu

**Signaling**
- JAK-STAT
- MAPK
- PI3K

- Proliferation, cytotoxicity
IL-2 Signaling
Cytokines that share the common gamma chain receptor
Where do cytokines come from?
Will cancer come to an end?
Which came first, the T cell or NK cell?

A BRIEF HISTORY OF IL-2

High Dose Interleukin-2
Kidney Cancer

Probability of Continuing Response

Complete Response (n=17)
Overall Response (n=43)
Partial Response (n=26)

Duration (months)
(10 Years)
Pioneering NCI studies

Biology/source
- T cell growth factor
- Jurkat source
- Recombinant E. coli

Preclinical models
- DLTs due to CLS
- Toxicities vary by species
- Dose-dependent activity

Early clinical studies+LAK

Role of IL-2 in adoptive cell-Rx strategies

Extramural IL-2 studies

In solid tumors
- With LAK cells
- Single agent
- With α-IFN
- With other cytokines
- With chemotherapy
- Toxicity modulation

- In heme malignancies
  - Trial methodology challenging
  - Phase II data promising
  - Phase III data disappointing
# High-dose IL-2 in advanced melanoma-270 patients

<table>
<thead>
<tr>
<th>Response</th>
<th>No.</th>
<th>%</th>
<th>Median</th>
<th>Range</th>
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<tbody>
<tr>
<td>CR</td>
<td>17</td>
<td>6</td>
<td>Not yet reached</td>
<td>3-158+</td>
</tr>
<tr>
<td>PR</td>
<td>26</td>
<td>10</td>
<td>5.9</td>
<td>1.5-91.5</td>
</tr>
<tr>
<td>CR + PR</td>
<td>43</td>
<td>16</td>
<td>8.9</td>
<td>1.5-158+</td>
</tr>
</tbody>
</table>

- Median survival for all responders: 62+ mo
- 29% of responders progression-free at 9 years

Durability of responses to high-dose IL-2 in melanoma
# Severe toxicities of high-dose IL-2

<table>
<thead>
<tr>
<th>Grade 3 or 4 Toxicity</th>
<th>Incidence</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>N = 255 (% of patients)</td>
</tr>
<tr>
<td>Hypotension</td>
<td>74</td>
</tr>
<tr>
<td>Pulmonary (dyspnea)</td>
<td>17</td>
</tr>
<tr>
<td>Renal (creatinine elevation)</td>
<td>14</td>
</tr>
<tr>
<td>Hepatic (hyperbilirubinemia)</td>
<td>21</td>
</tr>
<tr>
<td>CNS</td>
<td>32</td>
</tr>
<tr>
<td>Myocardial injury (ischemia, infarction, myocarditis)</td>
<td>6</td>
</tr>
<tr>
<td>Arrhythmias (all grades)</td>
<td>14</td>
</tr>
<tr>
<td>Infection</td>
<td>6</td>
</tr>
<tr>
<td>Thrombocytopenia</td>
<td>21</td>
</tr>
<tr>
<td>Death</td>
<td>4</td>
</tr>
</tbody>
</table>
IL-2 Conclusions ~1985-2000

- 15-20% pts w/RCC, melanoma benefit
- Rx ratio not improved by
  - IL-1 receptor agonist (decoy)
  - TNF blockade (Ab or decoy)
  - Lysophylline (lipid mediators)
  - Histamine (inhibit mφ ROS)
  - iNOS blockade (inhibit CLS)
- Dose-response inconclusive
- Not effective in biochemo
  - RCC w/pyrimidines, vincas
  - Melanoma w/DTIC, CDDP
- Novel strategies did not improve therapeutic index
  - With IFN α or γ
  - With tumor-directed Ab
  - With agonistic OKT3 Ab
  - Structure-function alterations
    - PEG-IL-2
    - Liposomal IL-2
    - IL-2 “specific agonist”
    - Albuleukin

Worth pursuing in RCC, melanoma, ?heme
IL-2: 2001-2007 and beyond

- Structural alterations
- Toxicity modulating agents
- Rational combinations hold promise for improving therapeutic ratio
  - Anti-vascular Rxs, small molecules, cytotoxics
  - Unique toxicities need further understanding
- ↑ insight into mechanisms
  - Host-immunogenetics, pharmacodynamics
  - Tumor (“Select” trial to validate CA-IX in RCC)
Interleukin-4

- Pleomorphic cytokine signals through STAT 6
- Th$_2$ cytokine mediates T-B, other interactions
- Net effects depend on cytokine and cell milieu
  - Mainly a B cell-stimulatory cytokine
  - Inhibits non-specific NK activity
  - Enhances other adaptive immune functions
    - Growth factor for Th2
    - Promotes proliferation and cytotoxicity of CTL
    - Stimulates MHC class II expression
    - Contributes to DC maturation
    - Enhances mΦ tumorcidal activity
IL-4 Signalling
Interleukin-4

- Promising data/proof of principle in Xgenic tumor (prototype for the GVAX strategy)
- Clinical experience limited
  - Studied like IL-2
  - Unfavorable therapeutic index
- Most promise as Rx to “elicit” moDC from PBMC
  - Used ex vivo w/GM-CSF
  - ? Comparison w/IL-13
IL-4 and IL-13

**Similarities**
- Predominantly anti-inflammatory effects
- Favor Th₂ responses
- Partially common receptor
- Promotes Ig class switch
- Used w/ GM-CSF → moDCs

**Differences**
- IL-13 activity on monocyte/mΦ cells
- IL-13 lacks B, T cell effects
- **IL-13 receptors on tumor cells, especially glioma**
  - Immunotoxins
  - Chimeric T cell Ag receptor

Assortment of receptor subunits depend on cell type
IL-6 is a very pleiotropic cytokine that plays a role in various biological processes including inflammation, immunity, reproduction, hematopoiesis, neural development, and bone metabolism. It induces the differentiation of T-cells, acute-phase reactants, nerve cells, B-cells, myeloma-plasmacytoma growth, and leukemic cell differentiation. It also affects cytotoxic T-lymphocytes, hepatocytes, astrocytes, plasma cells, and macrophages.
IL-6

- **Tumor source**
  - Unfavorable prognostic factor in renal CA, melanoma
  - An important growth factor for myeloma
  - Major effector of paraneoplastic thrombocytosis

- **Adaptive system**
  - B cell growth/differentiation
  - CTL differentiation
  - Type 2 responses

- **Preclinical data showed activity in selected tumor models**

- **Phase I and II clinical data**
  - Hematologic (thrombocytosis, anemia), arrhythmias, neurotox
  - Insufficient clinical activity
  - Concern about potential tumor-promoting effects

- **Paradox: IL-6 Ab now in clinical trials**
IL-7

Signaling
Jak1, Jak3/STAT5
PI3K activation, mTOR activation
IL-7 signaling and/or T-cell activation → receptor downregulation

Opposite regulation compared to IL-2, IL-15
IL-7 accumulates during lymphopenia as a result of diminished utilization
Homeostatic expansion of naïve cells during lymphopenia
Can substitute for IL-15 for homeostatic expansion of memory cells during lymphopenia

IL-7Rα expression marks cells destined to become memory during the evolution of the immune response
(Kaech, Nat Imm 2003)
IL-12

- Link between innate, adaptive immune response
  - Receptors on variety of immune cells
  - Prototypical type I cytokine, induces IFN-γ

- Potent inducer of counterregulatory type 2 cytokines
  - Emerged in clinical trials for advanced malignancy
  - Schedules and doses may be manipulated

- Clinical potential
  - Vaccine adjuvant
  - Induction of anti-angiogenesis
  - In combinations e.g. w/α-IFN, IL-2?
IL-12 and innate/adaptive immune system

IL-12 family
Jak/Stat Signaling: IL-12 versus IL-2
IL-15 and IL-2 compared

- **IL-2**
  - Activated T, B express high-affinity $\alpha\beta\gamma$
  - Prolif/differentiation NK, T, B
  - Elimination self-reactive T (AICD)
  - Maintenance Treg
  - +/- has autoimmune phenotype

- **IL-15**
  - Produced by DC, mono
  - Surface-bound on DC/mono $\leftrightarrow$ receptors on NK, CD8a1 cells
  - Promotes prolif NK, T, B, memory CD8
  - Inhibits AICD
  - +/- is lymphopenic for same populations
IL-18

- Member IL-1 family with IL-18BP counterregulation
- Activates NK cells and induces type I cytokines
- Promotes Th1 and memory CD8 T cells
- Upregulates FasL on effector lymphocytes
- Antitumor activity in:
  - Alone
  - W/IL-2, IL-12
- Phase I DLTs
  - Leukopenia
IL-21: another pleiotropic $\gamma_c$ cytokine

- **Durable anti-tumour activity**
  - Clonal expansion/proliferation
  - Increased effector function
  - Cytotoxicity
  - Cytokine production (IFN-$\gamma$, TNF-$\alpha$, IL-10)
  - Increased granzyme A/B, perforin
  - Inhibition of IL-15 induced proliferation

- **Humoral immunity**
  - B cell proliferation (CD40)
  - Increased IgG production
  - Decreased IgE production (IL-4)
  - B cell growth inhibition (IgM)

- **Anti-tumour activity**
  - Increased IFN-$\gamma$, IL-10, TNF-$\alpha$ production
  - Increased granzyme A/B, perforin
  - Increased cytotoxicity
  - Decreased growth
Phase I i.v. outpatient IL-21

J. Thompson et al, ASCO 2006

IL-21 Treatment Schedule
(outpatient administration of two 5-day cycles)

- Tolerable outpatient regimen identified
- Multiple dosing cycles feasible
- IL-21 pharmacodynamic activity
  - Direct effect on lymphocyte count
  - Increase in sCD25
- Four responses observed at different dose levels
  - One patient with Complete Response
  - Three patients with Partial Response

Phase II studies planned
RCC w/TKI (Phase I/II); Melanoma as SA
Thank you
[and to Jared Gollob]

Any questions?