The Journal of Immunology

IN THIS ISSUE

J Immunol 2006; 177:1-2; ;
doi: 10.4049/jimmunol.177.1.1
http://www.jimmunol.org/content/177/1/1

The Journal of Immunology is published twice each month by
The American Association of Immunologists, Inc.,
1451 Rockville Pike, Suite 650, Rockville, MD 20852

Print ISSN: 0022-1767 Online ISSN: 1550-6606.

THE JOURNAL OF IMMUNOLOGY

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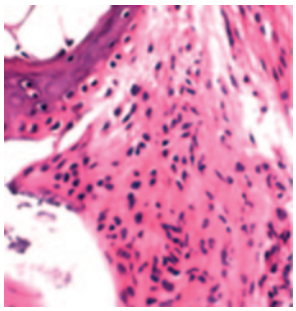
Aire-Independent Central Tolerance



Expression of peripheral Ags by medullary thymic epithelial cells (mTECs) facilitates central tolerance to prevent autoimmunity. Although the autoimmune regulator, aire, controls transcription of many genes, it is possible that aire-independent gene transcription occurs. Chin et al. (p. 290) measured abundant mRNA expression of type II collagen (CII) in thymii of 4-wk-old wild-type and *Aire*$^{-/-}$ C57BL/6 (B6) mice. *CII* mRNA expression was significantly lower in thymii and individual mTECs from B6 mice lacking lymphotoxin *(lta)* or its receptor *(ltbr)* than wild-type controls. *Aire* and *CII* mRNAs were elevated at 8 h in wild-type and *lta*$^{-/-}$ mice treated with an agonistic anti-LTβR mAb but returned to background by 24 h. Immunofluorescence localized CII protein in epithelial cells of the thymic medulla of wild-type and *Aire*$^{-/-}$, but not *lta*$^{-/-}$, mice; CII and aire proteins did not colocalize. Both *lta*$^{-/-}$ and *ltbr*$^{-/-}$ mice spontaneously developed high titers of anti-CII Ab, as did *Rag1*$^{-/-}$ recipients of *ltbr*$^{-/-}$ splenocytes or *ltbr*$^{-/-}$ splenic T cells plus wild-type B cells. Thymectomized B6 recipients of *ltbr*$^{-/-}$ thymi depleted of bone marrow-derived cells plus wild-type bone marrow also developed higher anti-CII Ab levels than controls. Mild symptoms of arthritis developed in wild-type B6 mice, but rapid collagen-induced arthritis appeared in *lta*$^{-/-}$ mice only after immunization with CII in CFA. CD4$^+$ T cells from CII-immunized *lta*$^{-/-}$, but not wild-type, mice responded vigorously to CII Ag in in vitro proliferation assays. The experiments demonstrate that collagen-induced arthritis induction is controlled by central tolerance through ectopic expression of CII in mTECs mediated by LT, not aire, in this mouse model of rheumatoid arthritis.

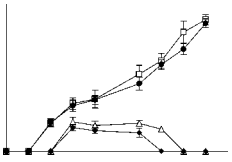
Defining Hemopoietic Stem Cells

Hemopoietic stem cells (HSCs) are self-renewing and differentiate into all blood cell lineages. However, there are few details regarding their cell cycle kinetics. Nygren et al. (p. 201) found that 100% of 14.5-day fetal liver HSCs had proliferated within 48 h. BrdU incorporation showed that more than half of HSCs were in G_1, compared with less than one-fourth of fetal liver hemopoietic progenitor cells (HPCs). Additionally, 14% of HSCs but no HPCs were in G_0. A slower turnover rate of HSCs compared with HPCs was confirmed by a time course study of in vivo BrdU incorporation that showed cell cycle transit times in fetal liver of 10.6 and 5.6 h, respectively. Isolated G_0-G_1 fractions were enriched over S-G_2-M fractions for multilineage long-term HSC (LT-HSC) repopulating activity when transplanted in lethally irradiated congenic adult recipients. The G_1 population from a primary or secondary ex vivo expansion of HSCs was highly enriched in LT-HSC repopulating activity; repopulating activity of the S-G_2-M population was due to contaminating short-term HSCs and LT-HSCs. HSCs that incorporated a high level of a label during division ex vivo (slowly dividing cells), but not HSCs that incorporated a low level of the label (rapidly dividing), had LT-HSC reconstitution potential after transfer into lethally irradiated recipients. The data indicate that proliferating LT-HSCs accumulate in G_1. Their prolonged transit through G_0-G_1 defines hemopoietic stem cells and may be a requirement for self-renewal.

Central Tolerance in Type 1 Diabetes

Proinsulin-2 expression is known to lower susceptibility to type 1 diabetes in humans and mice. Although expression of preproinsulin (proins-2) in medullary thymic epithelial cells in mice suggests central tolerance, lack of peripheral tolerance has not been established. Faideau et al. (p. 53) demonstrated proliferation and IFN-γ production by *proins-2*$^{-/-}$ CD4$^+$ T cells after proins-2 stimulation in vitro; wild-type cells were nonreactive regardless of parental origin of the *proin-2* gene. CD4$^+$ T cells from irradiated *proins-2*$^{-/-}$, but not wild-type, mice made chimeric with wild-type or *proins-2*$^{-/-}$ bone marrow produced IFN-γ in response to proins-2 in vitro. Cells from wild-type or *proins-2*$^{-/-}$ bone marrow chimeras with an engrafted *proins-2*$^{-/-}$ thymus in a thymectomized wild-type host also produced IFN-γ upon stimulation in vitro, although no islet infiltration or insulin Abs were detected in vivo. CD4$^+$ T cells from CD3ε-immunodeficient mice expressing proins-2 in their islets did not demonstrate in vitro Ag reactivity 2 mo after adoptive transfer with *proins-2*$^{-/-}$ CD4$^+$ T cells unless the mice were immunized with proin-2 at 2 mo. However, wild-type recipients of unprimed *proins-2*$^{-/-}$ cells developed Ag-reactive cells after in vivo immunization with the Ag, as did chimeras doubly engrafted with wild-type plus *proins-2*$^{-/-}$ thymii that received unprimed T cell-depleted wild-type bone marrow. The authors use bone marrow and thymus chimeras to show that central tolerance to proins-2 is conferred by expression of a single *proins-2* allele in thymic epithelium.

Tumor Escape Mutants



One challenge to successful immunotherapy to treat cancer is the development of tumor mutants resistant to CTLs. Chen and Ksander reported that mice immunized against murine mastocytoma cells were protected against a tumor challenge in the flank but not in the anterior chamber of the eye. In a continuation of their study, Chen et al. (p. 162) determined that tumor cells grown in the ocular chamber of immune competent or SCID mice formed tumors when injected in the flank of wild-type tumor-immunized animals. The immune escape phenotype was fully developed after 7 days of intraocular growth and was retained after 20 passages in vitro. Wild-type tumor cell-stimulated CTLs from wild-type tumor-immunized mice lysed ^{51}Cr-labeled wild-type,

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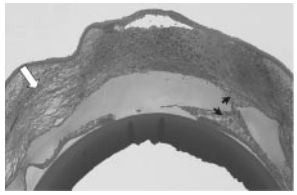
but not eye-derived, tumor cells in vitro. Restimulation of the CTLs with irradiated eye-derived tumor cells before the chromium release assay did not promote cytolytic activity. Proliferation of draining lymph node cells from immunized animals induced by eye-derived tumor cells was less than that induced by wild-type tumor cells not exposed to the ocular environment. No difference in expression of MHC class I or ICAM-1 molecules was detected between wild-type and eye-derived tumor cells stimulated with IFN-γ. Forty percent of mice immunized with eye-derived tumor cells were protected against tumor development after challenge with wild-type tumor cells, but 60% were protected after challenge with eye-derived tumor cells. The authors show that an immune escape phenotype rapidly develops in tumor cells in the anterior chamber of the mouse eye in the absence of selective T cell pressure and suggest that it is due to epigenetic, not genetic, changes induced by the ocular environment.

Resisting *P. aeruginosa* keratitis

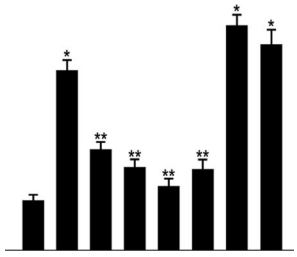


Cornea infection in extended-wear contact lens users and immunocompromised patients can be caused by the bacterium *Pseudomonas aeruginosa*. In a mouse model of the disease, the bacterium destroys the cornea of susceptible C57BL/6 (B6) mice but not resistant BALB/c mice. Huang et al. (p. 548) hypothesized that the negative regulator of TLR signaling, SIGIRR (single Ig IL-1R-related molecule), might be involved in keratitis induced by *P. aeruginosa*. *SIGIRR* mRNA expression was down-regulated from low constitutive levels in corneas of both strains of mice 12 h postinfection (p.i.); its up-regulation beginning at 1 day p.i. was much greater in BALB/c mice. SIGIRR protein levels were decreased in both infected strains at 1 day p.i. but increased at 3 and 5 days p.i. in BALB/c over B6 corneas. BALB/c mice that received anti-SIGIRR Ab had more corneal opacity and swelling, higher bacteria counts, and increases in *IL-1β*, *IL-1R*, *TLR4*, *IL-18*, *IFN-γ*, and *MIP-2* mRNA levels than controls. *IL-1R1*, *TLR4*, proinflammatory cytokine, and type-1 immune response-associated cytokine mRNA levels were reduced in LPS- or influenza virus protein-stimulated BALB/c monocytes/macrophage cells transfected with a plasmid expressing SIGIRR. IL-1-mediated NF-κB activation was reduced by cotransfection of the SIGIRR-expressing plasmid plus IL-1R1 into mouse cells carrying an NF-κB reporter plasmid. LPS-induced NF-κB activation similarly was inhibited by transfection of the SIGIRR-expressing plasmid into cells carrying a TLR4 signaling complex. The data indicate that the role of SIGIRR in resisting *P. aeruginosa* infection of corneas in BALB/c mice involves negative regulation of type-1 responses and IL-1R1 and TLR4 signaling.

Treg Homeostasis Indexed to IL-2

Maintenance of absolute numbers and relative sizes of populations of immune cells is necessary to prevent deregulation and disease. The Freitas laboratory showed that $CD4^+CD25^+$ regulatory T cells (Tregs) control peripheral $CD4^+$ T cell homeostasis and implicated IL-2 and its receptor. In a continuation of their work, Almeida et al. (p. 192) transferred varying proportions of Treg and naive T cells into T cell-deficient hosts; similar numbers of Treg cells were recovered from recipients 8–10 wk later. A constant fraction (10%) of Treg cells was obtained from irradiated $Rag2^{-/-}$ mice transferred with varying mixtures of bone marrow from wild-type and $CD25^{-/-}$ mice; $CD25^+$ bone marrow cells able to use IL-2 expanded preferentially in the peripheral $CD4^+$ T cell pool. $IL\text{-}2^{-/-}$ mice injected with mixed bone marrow from $CD25^{-/-}$ and $IL\text{-}2^{-/-}$ donors, but not with bone marrow from $IL\text{-}2^{-/-}$ donors alone, generated Tregs expressing *foxp3* mRNA at levels similar to wild-type Tregs. Experiments using varying mixtures of bone marrow in $Rag2^{-/-}IL\text{-}2^{-/-}$ recipients showed that only mice receiving an αβT cell source of IL-2 ($CD25^{-/-}$ bone marrow) developed Treg cells and were protected against death from autoimmune inflammatory bowel disease. $IL\text{-}2^{-/-}$ Tregs expanded significantly in $Rag2^{-/-}IL\text{-}2^{-/-}$ hosts when cotransferred with $IL\text{-}2^+CD4^+$ T cells but not when transferred alone or with $IL\text{-}2^{-/-}CD4^+$ T cells; the fraction and number of wild-type Tregs were higher when cotransferred with naïve $IL\text{-}2^+$ vs $IL\text{-}2^{-/-}$ T cells. Cotransfer of Tregs prevented inflammatory bowel disease in $Rag2^{-/-}$ hosts transferred with naïve $CD4^+$ T cells alone. The data show that peripheral expansion and survival of Tregs in mice is indexed to the number of $CD4^+$ T cells producing IL-2.

Preventing HIV-1-Induced Neurotoxicity



Mixed-lineage kinase 3 (MLK3) is thought to have a role in the pathogenesis of Parkinson's disease. Two neurological manifestations of HIV-1 infection, HIV-1-associated dementia and minor cognitive/motor disease, have symptoms similar to Parkinson's disease; an MLK3 inhibitor protects rat neurons from the toxic effects of HIV-1 glycoprotein 120 (gp120). Sui et al. (p. 702) found rapid phosphorylation of MLK3 and induction of apoptosis after exposure of primary rat neurons to HIV-1 proteins Tat (transactivator of transcription) or gp120; either of two MLK3 inhibitors prevented MLK3 activation and apoptosis. Tat- or gp120-induced neuronal apoptosis did not occur in cells transfected with a dominant negative MLK3 expression vector. JNK and p38 MAPK, two kinases downstream of MLK3, were phosphorylated in cells treated with Tat, and treatment of neurons with either a JNK or p38 MAPK inhibitor prevented cell death. Human monocytes treated with Tat or gp120 released high levels of TNF-α, as measured by ELISA, and activated p38 MAPK and JNK; MLK3 inhibitors prevented the cytokine release and kinase activations. However, only p38 MAPK, but not JNK, inhibitors abrogated Tat- or gp120-induced TNF-α production by monocytes. The data confirm that MLK3 is involved in HIV-1-associated dementia and that MLK3 inhibitors prevent HIV-1 Tat- or gp120-induced apoptosis of primary rat neurons and human monocytes. Tat or gp120 induces phosphorylation of both p38 MAPK and JNK in neurons, but of only p38 MAPK in monocytes.

Summaries written by Dorothy L. Buchhagen, Ph.D.