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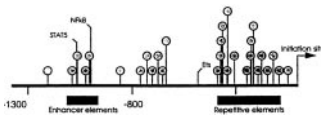
IN THIS ISSUE

T-bet

Expression of the transcriptional activator T-bet is induced during differentiation of naive CD4⁺ T cells into Th1 cells following exposure to Ag and IL-12. To establish the signaling pathway leading to T-bet induction, Takeda et al. (p. 4886) explored the interaction of IL-27 with WSX-1, an IL-12R-homologous class I cytokine receptor. Direct interaction of WSX-1 and JAK1 was demonstrated by their coprecipitation by an anti-JAK1 Ab. An in vivo phosphorylated WSX-1 construct also coprecipitated with STAT1 from lysates of cells transfected with the construct. STAT1 production and T-bet expression from IL-27-stimulated naive CD4⁺ T cells from WSX-1^{-/-} mice was negligible compared with the levels of STAT1 and T-bet detected in IL-27-stimulated WSX-1^{+/-} CD4⁺ T cells. IL-27 did not induce IFN- γ production in CD4⁺ T cells from either strain of mice, indicating that IL-27 induced T-bet in the absence of IFN- γ signaling and before interaction of cells with IL-12. RT-PCR analysis of the IL-27-stimulated WSX-1^{+/-} CD4⁺ T cells showed the induction of IL-12R β 2 whereas IL-27-stimulated WSX-1^{-/-} CD4⁺ T cells lacked IL-12R β 2 induction. The findings suggest that the IL-27/WSX-1 signal is a key Th1 initiating step in naive CD4⁺ T cell commitment.

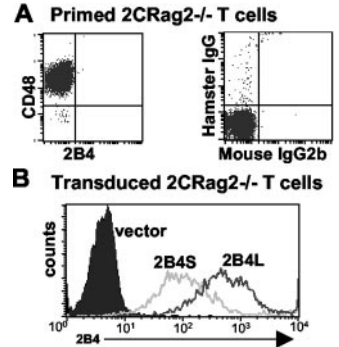
Perforin regulation by methylation

DNA methylation of upstream promoter sequences is an effective means by which the cell alters chromatin structure and suppresses gene expression. Lu et al. (p. 5124) found that treatment of PHA-stimulated CD4⁺ T and CD8⁺ T cells with the methyltransferase inhibitor 5-azacytidine resulted in a 4-fold increase in perforin mRNA expression as measured in oligonucleotide microarrays. Analysis by flow cytometry of CD4⁺ T and CD8⁺ T cells labeled with an anti-perforin Ab showed that perforin protein expression increased with the increase in gene transcription. The methylation pattern within the promoter region of the perforin gene was analyzed by sequencing PCR-amplified products of bisulfite treated DNA. The distal (-1326 to -650 bp) region of the promoter was more heavily methylated in CD4⁺ T than in CD8⁺ T cells whereas the region from -300 bp to the transcription start site was hypomethylated in both cell types. Demethylation by treatment with 5-azacytidine was most prominent in the enhancer-containing region between -900 and -600 bp. The results indicate that methylation between the upstream enhancer at -1000 bp and the distal promoter at -300 bp plays a role in regulating perforin gene expression in T cells.



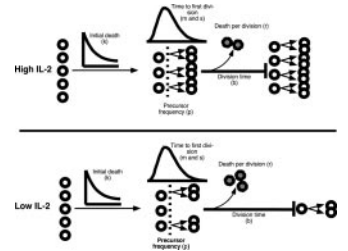
Enhancing T cell cytotoxicity

The NK cell receptor 2B4, a member of the Ig superfamily, is expressed as a long form (2B4L) and a short form (2B4S) in murine NK cells and binds with CD48. 2B4 is also found in 1–2% of splenic CD8⁺ T cells from mice. To explore the function of 2B4 in CD8⁺ T cells, Lee et al. (p. 4881) introduced the two isoforms of 2B4 by retroviral transduction into CD8⁺ T cells having a defined TCR (2C/Rag2^{-/-}) that recognized L^d class I molecules. Both the 2B4L and 2B4S transductants lysed target P815 mastocytoma cells presenting CD48. Surprisingly, the transductants had greater lytic activity against the CD48-negative P815 cells. Transduction of 2B4L or 2B4S into cell lines that did or did not express L^d demonstrated that lysis was MHC-restricted and that the lysis of the L^d-expressing cells was augmented by 2B4. The 2B4-enhanced cytotoxicity was reduced by the addition of anti-CD48 mAbs during the transduction of the T cells with 2B4L or 2B4S and was almost totally blocked by continuing the mAb incubation during the ⁵¹Cr release assay. The authors' interpretation was that the 2B4/CD48 interactions took place before target cell exposure in a novel form of costimulation among neighboring T cells. The ability of the 2B4/CD48 interaction to increase cytotoxicity of CD8⁺ T cells could be useful in facilitating anti-tumor immunity.



Six parameters may be better than four

Interleukin-2 has a critical role in T cell proliferation but there is a difference in the amount required in vitro compared with in vivo. Deenick et al. (p. 4963) developed a stochastic model to examine the effects of different concentrations of IL-2 on the T cell response. Their model refines the previous four-parameter model of Gett and Hodgkin. The new model adds two additional parameters that take into account the precursor frequency of cells that enter into the first cell division and the death rate for cells in cell division 1 or greater. The predictions of the new model were verified experimentally. IL-2 concentrations did not alter the time to first division, but higher subsequent division rates and lower death rates were observed in T cells exposed to higher concentrations of the cytokine. The rate



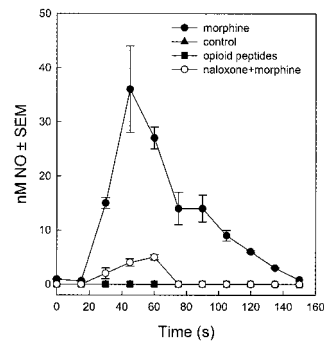
of cell death in culture was unaffected by IL-2 concentration up to 48 h. Beyond that time, only cells cultured in high concentrations of IL-2 began to divide as measured by incorporation of TdR. If T cells stimulated in culture with anti-CD3 did not receive IL-2 within 24 h, they lost the ability to divide and could not be rescued by later addition of IL-2. The six-parameter model illustrates how small changes in each parameter can induce large changes in cell numbers and reconciles some of the paradoxes between the in vivo and in vitro contributions of IL-2 to T cell proliferation.

Morphine and immunity

The possible role of morphine as an endogenous signaling molecule in cells of the immune system has been strengthened by the paper of Cadet et al. (p. 5118). The authors cloned the full-length (1338 bp) human μ_3 opiate receptor subtype that the group previously had characterized by ligand binding and biochemical activity. Their probe was a μ opiate receptor gene-specific DNA generated by PCR of a human testis cDNA library. The μ_3 clone had 100% DNA sequence identity to μ_1 in the center and conserved region but had several hundred nucleotides spliced out at the 5' end. The 3' end was comprised of three sequences: one from the 3' end of the μ_1 receptor gene, followed by a new fragment of 263 bp, and terminating with a 202 bp fragment of the 3' end of the μ_1 gene untranslated region. The μ_3 mRNA transcript was detected by Northern analysis in human heart endothelial tissue, in human leukocytes and in COS-1 cells transfected with the μ_3 cloned cDNA. The μ_3 receptor in the transfected COS-1 cells had functional properties previously demonstrated, namely selectivity for opiate alkaloids and insensitivity to opioid peptides. These findings may contribute to an understanding of the interactions between the immune and nervous systems.

IL-15R isoforms

The existence of a novel IL-15R on mast cells has been postulated to resolve the contradictory results reported for the action of IL-15 on those cells. On p. 5045, Bulanova et al. describe the existence of three novel isoforms of the IL-15 receptor α -chain (IL-15R α) in bone marrow-derived mast cells from mice. These isoforms were obtained by RT-PCR of IL-15 α mRNAs that were cloned in an expression vector. DNA sequence analysis revealed that the three isoforms lacked exons 3, 3 and 4, and 3, 4, and 5, respectively, of the IL-15R α gene. The splicing patterns are different from those of four alternatively spliced IL-15R α variants that had been reported in human tissues. Bulanova et al. transfected the mouse alternatively spliced isoform clones into several mast cell lines. The protein products associated primarily with the



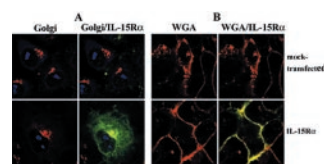
Golgi apparatus and the plasma membrane, bound IL-15, and phosphorylated STAT5, STAT6, Jak2 and Syk. Cell lines stably expressing the IL-15R α isoforms were protected against IL-15 induced apoptosis. The identification of three functional IL-15R α isoforms on primary mast cells in mice suggests that IL-15 might be an allergic cytokine and offers the possibility that IL-15 antagonists can be developed to block mast cell activities.

Majoring on cytokines

Murine infection with the intracellular parasite *Leishmania major* has been used as a model to study the role of Th subset development in infectious disease. Th1 development is associated with control of infection, whereas Th2 development is associated with disease progression. Using this model, it has been possible to study the role of various cytokines in the Th subset differentiation process. On p. 5152, Noben-Trauth et al. present studies exploring the role of IL-10 in the susceptibility of IL-4R $\alpha^{-/-}$ mice to various strains of *L. major*. IL-4R $\alpha^{-/-}$ mice were previously shown to be susceptible to infection with the LV39 isolate of *L. major*. The authors found that lesion sizes and parasite numbers were dramatically reduced in both anti-IL-10R mAb-treated IL-4R $\alpha^{-/-}$ mice, as well as in IL-10 $^{-/-}$, IL-4R $\alpha^{-/-}$ double-knockout mice infected with the LV39 strain. In addition, the authors used the IR173 strain of *L. major* to show that although IL-4R $\alpha^{-/-}$ mice effectively control infection with this strain, anti-IL-10 mAb treatment resulted in a further reduction in parasite number. These studies show that, in addition to IL-4/13, IL-10 is an important factor in promoting disease in the *L. major* model.

IL-15 and survival

During the immune response to a pathogen, naive T cells are stimulated to proliferate and differentiate into effector cells following encounter with antigen. After elimination of the pathogen, most effector cells undergo cell death, though some remain in the host to become memory cells. Memory T cells exhibit much higher rates of proliferation and death than naive T cells. The turnover of memory cells can be influenced by a number of compounds, including certain cytokines. Berard et al (p. 5018) examined the effect of IL-15 on the proliferation and survival of naive (CD44^{low}) and memory (CD44^{high}) CD8⁺ T cells in vitro. They found that while preferentially promoting proliferation of memory CD8⁺ T cells, IL-15 rescues both naive and memory CD8⁺ T cells from apoptosis. This enhanced survival correlated with up-regulation of Bcl-2 in both subsets of cells following IL-15 treatment and up-regulation of Bcl-x_L in the memory cells. The authors also examined which chains of the IL-15R complex are required for proliferation and survival of CD8⁺ T cells. Using IL-15R α gene knockout mice, they found that the α -chain of the receptor is largely dispensable for proliferation and survival in response to IL-15, though it did enhance the sensitivity of cells to low doses of the cytokine. In contrast, both the β - and γ -chains of IL-15R were shown, using anti-IL-2R β and anti- γ C Abs, to be necessary for the proliferation and survival of CD8⁺ T cells in response to IL-15. These studies indicate that IL-15 exerts differential effects on the turnover of both naive and memory CD8⁺ T cells.



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