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upstream of exon 1a. The proximal promoter has multiple transcription start sites, but the distal promoter only has a single start site. Culturing NK cells with IL-2 leads to a significant increase in the proportion of transcripts initiated from the distal promoter suggesting that the distal promoter may be more IL-2 responsive.
CD8+ αβ T cell clones were identified that were negative for cell surface and intracellular expression of CD94 protein, yet positive for CD94 transcripts. Transcripts with and without exon 1a were observed. CD94 cell surface expression was induced after culturing with IL-2, IL-15 and PHA but not in T cells that lacked CD94 transcripts, suggesting that CD94 protein expression in such T cells is controlled at both the transcriptional and post-transcriptional levels.
We identified three new alternatively spliced transcripts of CD94. One of these transcripts lacks part of exon 3 (T3) and another lacks exon 4 (CD94B-T2), while the third lacks part of exon 5 (T4) that encodes the ¡§neck¡¨ region of the protein. Protein expression studies show that T4 encoded protein preferentially dimerizes with NKG2B compared to NKG2A.

67.5

The proto-oncogene and Th2 transcription factor c-Maf modulates interleukin-10 gene expression in macrophages

Shanjin Cao, Xiaojing Ma. Microbiology and Immunology, Weill Medical College of Cornell University, 1300 York Avenue, New York, NY 10021

The appropriate balance between pro- and anti-inflammatory responses is essential for the establishment of homeostasis. Among the various mechanisms conducted to control the inflammatory processes, the activities of IL-10 play a fundamental and most important role. However, the molecular mechanisms whereby the expression of IL-10 gene is regulated in macrophages are poorly understood. Our study has identified the proto-oncogene c-Maf as a critical regulator of IL-10 gene expression. We have made the following novel observations: (1) c-Maf is constitutively expressed in macrophages. (2) IL-10 production stimulated by LPS in macrophages derived from c-Maf knockout mice is severely impaired. (3) Adenovirus-mediated c-Maf gene transduction into human macrophages can induce both the mRNA and protein expression of IL-10. (4) Overexpression of c-Maf in monocytic cells strongly induces IL-10 promoter activity. (5) The potential c-Maf response element has been mapped to within 129 bp upstream of the transcription start site of the human IL-10 promoter, where a c-Maf-induced perturbation of nuclear DNA-binding activity is noted. We are also investigating under which physiological (e.g. the establishment of homeostasis) and/or pathphysiological circumstances this transcriptional pathway is invoked.

This work is supported by National Institutes of Health Grants AI-45899 and CA-79772 (X.M.), and Susan G. Komen Breast Cancer Foundation Postdoctoral Fellowship (S.C).

67.6

Beryllium-induced gene changes in a mouse alveolar macrophage cell line

Melanie Sarah Flint, Sally S Tinkle. Toxicology & Molecular Biology Branch, CDC/NIOSH, 1095 Willowdale road, Morgantown, wv 26505

Chronic Beryllium Disease (CBD) is an occupationally acquired lung disease that occurs as a cell mediated immune response to beryllium, resulting in the development of noncaseating granulomas. We hypothesized that the identification of early genes associated with beryllium exposure may give important insights into the role of macrophages in CBD. We investigated gene expression in a mouse alveolar macrophage cell line incubated in the presence of absence of 10μM $BeSO_4$ for 4 hours. We utilized Affymetrix oligonucleotide arrays consisting of approximately 12000 genes to detect differential changes in gene expression in beryllium-treated cells compared to controls. We found that beryllium caused significant changes in the expression of numerous genes encoding; heat shock proteins, cytokines, adhesion molecules, cytokine receptors, signalling molecules and transcriptional activators and repressors. HSP70 and MHC class III region were elevated 10-fold in $BeSO_4$ treated macrophages. However, IL-1[beta], ICE, ICAM-1 and IL-10R were all decreased by beryllium. We verified beryllium-modulation of these genes by real time quantification (Taqman™PCR). Our studies identify beryllium-regulated genes and suggest that HSPs and cytokine genes play pivotal roles in an early immune response to beryllium. This work is supported by CDC/NIOSH.

67.7

Up-regulation of MHC Class II Transactivator (CIITA) expression in B lymphocytes by IFN-γ

Janet F. Piskurich[1], Carolyn A. Gilbert[1], Brittany D. Ashley[1], Jian Wu[2], Kenneth L. Wright[2]. [1]Department of Basic Medical Sciences, Mercer University School of Medicine, 1550 College St., Macon, GA 31207, [2]Department of Biochemistry and Molecular Biology, University of South Florida, Tampa, FL

Class II transactivator (CIITA), the master regulator of MHC class II (MHC II) expression, is a co-activator that controls MHC II transcription. Additionally, CIITA can activate MHC class I expression. B lymphocytes express MHC II constitutively because CIITA promoter III (pIII), one of the multiple promoters (pI-pIV) of this gene, is constitutively expressed. Increases in MHC II expression in B cells in response to cytokines have been observed. Although IFN-γ induces expression of MHC II and CIITA in many cell types, the effect of this cytokine on CIITA expression in B cells has not been previously studied. RT-PCR analyses demonstrate that MHC II and CIITA pIV-specific RNAs are increased in Raji B cells in response to IFN-γ treatment. Functional promoter analyses confirm that pIV is the IFN-γ-inducible promoter of CIITA in these cells and reveal that the IRF-1-binding site is required for induction. In vivo genomic footprint analysis demonstrates protections at the Stat1 and IRF-1-binding and E-box sites. Since B cells exhibit both constitutive transcription of CIITA via pIII and also inducible transcription via pIV, it may be possible to use IFN-γ to activate CIITA and MHC expression in myeloma cells where BLIMP-1 represses the activity of CIITA pIII.
This investigation was supported (in part) by research grants from the National Multiple Sclerosis Society and the MEDCEN Community Health Foundation of Central Georgia.

67.8

DNA Methylation and Chromatin Structure Suppress Perforin Expression in Normal T Cells, and Contribute to its Overexpression in CD4+ Lupus T Cells

Qianjin Lu[1], Ailing Wu[1], Mariana Kaplan[1], Mathias Lichtenheld[2], Bruce Richardson[1]. [1]Medicine, University of Michigan, 5431 CCGCB, Ann Arbor, MI 48109-0940, [2]Immunology, University of Miami, Miami, FL

The mechanisms regulating perforin expression are incompletely understood. Treating T cells with the DNA methylation inhibitor 5-azacytidine (5-azaC) increased perforin ~4-fold, so we examined the methylation and chromatin structure of its promoter and upstream enhancer. The entire region was unmethylated in NK cells and methylated in fibroblasts. In contrast, only the promoter was unmethylated in CD4+ and CD8+ cells, and expression correlated with hypomethylation of an area flanking the enhancer. 5-azaC selectively demethylated this area and increased perforin expression. Selective methylation of this region suppressed promoter function. Finally, perforin expression was associated with sensitivity of the region to DNase I digestion. CD4+ T cells from lupus patients have hypomethylated DNA, so we asked if they aberrantly express perforin. Normal CD4+ T cells did not express perforin, while 7-10% % of CD8+ cells expressed the protein. CD4+ T cells from patients with active lupus expressed perforin, and the number increased with disease activity. Expression correlated with demethylation of the same region affected by 5—azaC. In contrast, CD8+ T cells from patients with active lupus did not express perforin, and the methylation sensitive region was hypermethylated. Concanamycin A, a perforin inhibitor, suppressed lupus T cell killing of autologous monocyte by ~75% ($p<0.05$), indicating that the perforin expression was functionally significant. We conclude that DNA methylation and chromatin structure regulate perforin expression in T cells, and that abnormal DNA methylation contributes to aberrant perforin expression in CD4+ lupus T cells. AG014783, AR42525, and AI42753 and the VA.

The American Association of Immunologists
90th Anniversary Annual Meeting

Immunology 2003
Denver, Colorado
May 6 – 10, 2003

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Key Word
Author

The American Association of Immunologists

American Association of Veterinary Immunologists
American Society of Transplantation
Association of Medical Laboratory Immunologists
Canadian Society for Immunology
Clinical Immunology Society
International Society for Interferon and Cytokine Research
International Society for NeuroImmunoModulation
International Society of Neuroimmunology
PsychoNeuroImmunology Research Society
Society for Leukoctye Biology
Society for Mucosal Immunology
Society for Natural Immunity

THE FASEB JOURNAL

Volume 17, Number 7 April 14, 2003

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WEDNESDAY
May 7, 2003

THURSDAY
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FRIDAY
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Editor-in-Chief
Boyer Center for Molecular Medicine
Yale University School of Medicine
295 Congress Avenue, (P.O. Box 9812)
New Haven, CT 06519-1418, USA
Phone: 203-737-2334 Fax: 203-737-2267
email: vincent.marchesi@yale.edu

Editorial Office
Boyer Center for Molecular Medicine
Yale University School of Medicine
295 Congress Avenue (P.O. Box 9812)
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Phone: 203-737-2334 Fax: 203-737-2267
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Publications Office
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The FASEB Journal (ISSN-0892-6638) is published 15 times a year (monthly except three times in March and two times in April) by the Federation of American Societies for Experimental Biology, 9650 Rockville Pike, Bethesda, MD 20814-3998, U.S.A.

Periodicals postage paid at Bethesda, Maryland, and at additional mailing offices. **Postmaster:** Send change of address to *The FASEB Journal*, 9650 Rockville Pike, Bethesda, MD 20814-3998. The views expressed in articles are those of the authors and not necessarily those of the Federation. Send manuscripts and proposals to the Editor-in-Chief. See **Instructions for Authors** online at **http://www.fasebj.org.**

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