772.9

Signal Pathways Involved In the Inhibitory Effects of Prostaglandin E2 on Chemokine Production

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Exposure to pathogen induces dendritic cells (DC) to release inflammatory cytokines and chemokines and various agents control the inflammatory response. PGE2 has been shown to inhibit chemokine production from DCs through EP2 and/or EP4 signal pathways. In this study, we report on the inhibition of PGE2 on the LPS-induced CCL3 and CCL4 production by murine DCs. The inhibition is mediated through intracellular cAMP, since PGE2 induces high levels of cAMP in DCs and dibutiryl-cAMP exerts similar inhibitory effects on chemokine production. However, H89 does not reverse the inhibition of PGE2, suggesting that cAMP acts through a mediator different from PKA. PGE2 does not induce phosphorylation of CREB, nor does it affect LPS-induced phosphorylated CREB. ERK1/2 is important for LPS-induced chemokines, while PGE2 does not affect the ERK or other MAPKs. EMSA reveals that PGE2 does not affect the LPS–induced binding activity of NFkB and AP1. However, PGE2 changes the composition of the AP1 complexes, decreasing c-Jun and increasing JunB. Protein/DNA assays, which screen a large number of transcription factors (TF), reveal that PGE2 decreases the DNA binding of several TFs such as Sp1, NFAT and SRE, and that it induces the binding of CDP, a known transcription repressor. These results suggest that PGE2 inhibits chemokine production by inducing cAMP and affecting the activity of several TFs. Support: AI47325 and AI052306 to DG and Johnson & Johnson Fellowship to JH and EV.

772.10

Gene regulation of the Th1-specific and STAT4-inducible murine IL-18Rα gene

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The IL-18 receptor consists of two subunits, IL-18Rα and IL-18Rβ, which mediate synergy with IL-12 signals in the induction of IFNγ. IL-18Rα is expressed in Th1 but not in Th2, and expression is induced by IL-12 in a STAT4-dependent manner. Little is known about the transcriptional and epigenetic mechanisms controlling IL-18Rα gene expression. Three STAT4-binding sites are located within the intergenic region between the IL-1 receptor-like 1 precursor (IL1RL1) and IL-18Rα genes that specifically bind STAT4 in vitro and in vivo. STAT4-induced epigenetic modifications of chromatin at specific loci may mediate a Th1 gene expression program. Initial DNase I hypersensitivity (DH) analysis identified a constitutive DH site located in intron 1 of the IL-18Rα gene in Th1 and Th2 cells. Chromatin immunoprecipitation demonstrated that Th1 differentiation was accompanied by slightly increased acetylation of histone H3 within the IL1RL1-IL18Rα intergenic region. Using methylation sensitive restriction enzymes, we demonstrated that the transcriptional start site of IL-18Rα locus was hypomethylated in undifferentiated T cells and Th1 cells but became specifically methylated in Th2 cells after long-term culture. Since DNA methylation is associated with gene silencing, these results suggest that stable epigenetic changes at the IL-18Rα locus accompany Th cell differentiation. Supported by NIH RO1 AI45515.

772.11

Signaling Mechanism of Protein Kinase PKR in the Regulation of Cytokine Expression by Epstein-Barr virus Latent Membrane Protein 1

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The oncogenic Epstein-Barr virus latent membrane protein 1 (LMP1) has been known to have cellular transformation properties and it induces cytokine expression including TNF. We previously showed that TNF and interferon interact to induce cellular effects via the activation of an dsRNA-activated protein kinase PKR, a signal transducer that regulates MAPK activities and cytokine induction. We investigated whether PKR plays a critical role in LMP1-regulated cytokine expression. BJAB (EBV-negative B-lymphoma) cells were transfected with pcDNA3 encoding LMP1-sense cDNA. Here, we showed that LMP-1 expression induces the synthesis of IL-6, IL-10 and GM-CSF in the cells. Suppression of PKR by 2-aminopurine (2AP, a PKR-specific inhibitor) resulted in abrogation of the cytokine expression in these cells. There were concomitant decreases in phosphorylation levels of p38 kinase in these 2AP-treated, LMP1-expressing BJAB cells. The results suggest that PKR is upstream of p38K in the LMP1-induced signaling pathways. Additionally, we showed that transcription factor NF-kB was activated in the LMP1-expressing BJAB cells as compared to the control LMP1-negative BJAB cells. The NF-kB activation also was dependent on PKR activity. In summary PKR may play a critical role in mediating the effects of LMP1 on the immune system, via the induction of p38K and NF-kB. (Supported by grants to ASL from HK Research Grants Council)

772.12

Molecular characterization of chicken IL-16 and IL-17

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Two chicken cytokine genes homologous to mammalian IL-16 and IL-17 were identified from a chicken intestinal EST library and their biological properties were characterized. Chicken intestinal EST library was prepared from the intestinal IELs of Eimeria-infected chickens. Chicken IL-16 is predicted to be consisted of 607 amino acids and showed 86% sequence identity to duck IL-16, and 49 - 52% identity to various mammalian homologues. IL-16 mRNA were identified in several chicken lymphoid tissues but none of the non-lymphoid tissues examined. A recombinant chicken IL-16 protein which was expressed in COS-7 cells displayed chemoattractant activity for splenic lymphocytes. Chicken IL-17 contained a 507 bp open reading frame and is predicted to encode a protein of 169 amino acids with a molecular mass of 18.9 kDa which includes a 27 residue NH2-terminal signal peptide, a single potential N-linked glycosylation site, and 6 cysteine residues conserved with mammalian IL-17s. IL-17 shared 37 - 46% amino acid sequence identity to the previously described mammalian homologues and contained sequences homologous to the open reading frame of HVS 13. IL-17 transcripts were identified in a REV-transformed chicken CU205 cell line and Con A-stimulated splenic lymphocytes but not in normal tissues. Supernatant of COS-7 cells transfected with chicken IL-17 cDNA induced IL-6 production by chicken embryonic fibroblasts.

772.13

Role of cell/cell interactions in silica-induced production of inflammatory mediators in the lung

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Exposure of rat alveolar macrophages (AM) to silica *in vitro* upregulates mRNA expression of a limited number inflammatory mediators compared mRNA upregulation in AM isolated after *in vivo* exposure to silica (Rao et al., FASEB J. **17**:C294, 2003). This observation suggested that cell/cell interactions may play a role in upregulating mRNA expression of some inflammatory mediators. In order to study cell/cell interactions, we isolated AM, rat alveolar type II cells (type II) and lung fibroblasts (LF). mRNA expression of five genes (IL-1β, IL-6, IL-10, iNOS and GM-CSF) was measured by real-time RT/PCR following co-culture of AM with previously cultured type II cells or LF, with or without 200 μg/ml of silica (Min-U-Sil 5). Silica had no additional effects on the expression of any of the cytokines. Therefore, only the data with co-cultures alone, without silica exposure, are shown below:

	AM*	Type II*	Type II + AM*	LF*	LF +AM*
IL-1β	1.21 ± 0.07	0.03 ± 0.01	1.1 ± 0.06	N.D.	2.2 ± 1
IL-6	1.27 ± 0.15	0.06 ± 0.26	3.9 ± 3.16	36 ± 26	1730 ± 531
IL-10	1.21 ± 0.11	1.8 ± 0.5	1.4 ± 1.46	0.2 ± 0.3	27 ± 9
iNOS	1.36 ± 0.11	0.15 ± 0.02	1.7 ± 1.63	0.7 ± 0.4	11.5 ± 4
GM-CSF	1.33 ± 0.18	0.06 ± 0.18	3.5 ± 0.71	23 ± 11	40 ± 17

*The numbers in these columns represent mRNA expression (mean ± SEM of at least 3 separate experiments) relative to AM alone (column 2). N.D. not detectable.

These data indicate that LF are an important source of inflammatory mediators and that AM/LF interactions play a role in the expression of IL-6, IL-10 and iNOS in basal co-cultures.

772.14

The decoy IL-13 receptor downmodulates granulomatous inflammation and promotes host survival in chronic schistosomiasis

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For many helminth infections, downmodulating the immune response is critical because persistent inflammation can become more damaging to the host than the invading pathogen itself. Such is the case with schistosomiasis mansoni, where chronic granulomatous inflammation in the liver causes fibrosis, portal hypertension, and eventual death if not suppressed effectively. Our studies have shown that IL-13 is the primary cytokine that promotes liver fibrosis. Therefore, we sought to identify endogenous mechanisms that regulate IL-13, including the IL-13 receptor alpha 2 (IL-13Ra2). Mice deficient in IL-13Ra2 were percutaneously exposed to S.mansoni cercariae and disease progression compared to wild type (WT) BALB/c mice. Chronically infected IL-13Ra2-KO mice experienced increased mortality and an examination of liver pathology revealed larger granulomas. IL-13Ra2 deficiency was also associated with increased eggs in the lungs, evidence of increased portal hypertension as a consequence of exacerbated liver fibrosis which develops in these mice. Furthermore, soluble serum IL-13Ra2 was shown to be increased in chronically infected Ugandan patients living in an endemic area. These data indicate that IL-13Ra2 is a critical mediator of immune downmodulation in schistosomiasis, identifying the receptor as a new life sustaining "off" signal for chronic and pernicious inflammation.

REGULATION AND EVASION OF THE IMMUNE RESPONSE BY PATHOGENS (773.1-773.20)

773.1

Coxsackievirus B4 infection depletes thymocytes and up-regulates MHC class I expression in human thymus

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Coxsackievirus B4 (CBV4) infection has been implicated in the onset of autoimmune destruction of pancreatic β-cells, yet the mechanism is not known. Given immunological self-tolerance begins in the thymus, we investigated CBV4 infection within human fetal thymic organ cultures (FTOC). Herein we observe productive infection within FTOC by CBV4. CVB4 also induced a marked depletion of DP and CD4+ thymocytes. Viral RNA detection in those subsets and the absence of any enhanced apoptosis indicate that such depletion resulted from CVB4 infection. MHC I expression was also up-regulated on DP in FTOC after inoculation. This increase in MHC I was associated with infection as it increased in a dose dependent manner with viral innoculum and was inhibited by FTOC treatment with chloroquine. Although MHC I up-regulation can also occur from an INF-α response to viral challenge, FTOC treatment with anti-IFN-α neutralizing antibodies had no effect and little to no IFN-α could be detected in FTOC. To clarify this further we quantified IFN-α secreting plasmacytoid dendritic cells (pDC) within FTOC and observed significant depletion of pDC after 7 days. In conclusion, this study demonstrates that CVB4 infection of human FTOC severely interferes with intrathymic thymocyte development and circumvents the INF-α response. Such pathogenic mechanisms are likely to impact in a developing immune system.

773.2

Maintenance of Vaccine-Specific Central Memory CD4+ T Cells is Impaired in HIV Infection

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Protection following successful immunization is associated with the generation and maintenance of antigen-specific memory T cells. CD4+ T cell help is required for the generation of functional memory CD8+ T cells. While a strong HIV-specific CD4+ T cell response has been correlated with better control of infection, the effect of HIV on the maintenance of antigen-specific central memory CD4+ T cells is not fully understood. We characterized the function and phenotype of memory CD4+ T cells generated by measles, mumps, tetanus and influenza vaccinations in HIV infected individuals receiving anti-retroviral therapy (ARV; n = 15), long term non-progressors (LTNP; n = 10), and healthy seronegative volunteers (n = 8). We observed significantly decreased IL-2 production and proliferation of the CD28+/CCR7+/CD45RA- (central memory) CD4+ T cell population in the HIV infected individuals compared to the seronegative controls. In addition, antigen-specific proliferation correlated with the nadir CD4+ T cell count, but not with either the restored current CD4+ T cell count or with HIV viral load. Our results suggest that HIV infection leads to impaired maintenance of vaccine-specific central memory CD4+ T cells. These findings have important implications for the timing of ARV initiation, vaccine as well as immune reconstitution strategies.

773.3

STAT5 Regulates Activity of the HIV-1 LTR, Under Negative Feedback Control by Nef

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Eradication of HIV-1 by anti-retroviral agents is thwarted by the ability of the virus to "hide" in CD4 T lymphocytes. These latently infected T cells provide a viral reservoir for infection of new cells. We previously identified a critical signaling pathway, JAK3/STAT5, as one of the targets of HIV-mediated immune inhibition. Here, we investigated the hypothesis that inhibition of STAT5 activation suppresses the HIV-1 long terminal repeat. We identified 3 regions in the LTR as close matches to the STAT5 consensus-binding site, and show that STAT5 binds to the LTR in vivo during HIV-1 infection. This binding by STAT5 leads to activation of the LTR, even in primary resting CD4 T cells. Furthermore, we show that a nef deletion mutant of HIV-1 fails to inhibit JAK3 and STAT5, and that expression of Nef inhibits STAT5 transactivation. We propose that latency is, in part, the result of a negative feedback loop – transcription of HIV-1 leads to down-regulation of itself, via Nef inhibition of STAT5. An understanding of the mechanisms of induction of HIV-1 latency will lead to therapeutics designed to prevent or eliminate latent cells.

773.4

Reduced expression of Bcl-2 and Bcl-xL in HIV-specific CD8+ T cells; restoration by IL-15

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We recently demonstrated that HIV-specific CD8+ T cells are highly sensitive to spontaneous and CD95/Fas-induced apoptosis, and that IL-15 inhibits this apoptosis. In this study we examined the levels of anti-apoptotic molecules Bcl-2 and Bcl-xL in HIV-specific CD8+ T cells from HIV-infected individuals and the effect of IL-15 treatment on this expression. Bcl-2 expression was markedly decreased in HIV-specific (MFI= 219 ± 33) compared to CMV-specific (MFI= 336 ± 22) and total CD8+ T cells (MFI= 376 ± 32) from HIV-infected individuals as well as