07

Chemokines

07-02/P

UPREGULATION OF CCL20 AND RECRUITMENT OF CCR6+ GASTRIC INFILTRATING LYMPHOCYTES IN HELICOBACTER GASTRITIS

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Helicobacter pylori (*H. pylori*) infection is associated with an inflammatory response in gastric mucosa, leading to chronic gastritis, peptic ulcer and gastric cancer. There is increased T cell infiltration at the site of infection with *H. pylori*. CCR6, the specific β-chemokine receptor for CCL20 (MIP-3α/LARC/exodus), has recently been reported to mediate lymphocyte homeostasis and immune responses in mucosa tissue, and it may play a role in chemokine-mediated lymphocyte trafficking during gastric inflammation. In this study, we investigate the role of CCR6 and its ligand CCL20 in inducing inflammatory response in gastric mucosa during *H. pylori* infection. The gastric infiltrating T lymphocytes were isolated from endoscopic biopsy specimens of *H. pylori* gastritis patients and analyzed for the expression of chemokine receptors. Our results demonstrate that Th1 chemokine receptors CXCR3, CCR5, and CXCR6 but not Th2 chemokine receptor CCR3 were expressed on gastric infiltrating T lymphocytes. Meanwhile, there was significantly increased CCR6 expression in CD3+ T cells infiltrating the gastric mucosa; and its ligand, chemokine CCL20, was selectively expressed in inflamed gastric tissues. The production of CCL20 was upregulated in response to *H. pylori* in primary human gastric epithelial cells when stimulated by the proinflammatory cytokines IL-1 β and TNF-α. Furthermore, recombinant CCL20 induced lymphocyte chemotaxis migration in fresh gastric T cells in chemotaxis assay ex vivo, indicating that the gastric T cells could migrate toward inflammatory sites via CCR6/CCL20 interaction. Our results suggest that the interaction between CCL20 and CCR6 may play a role in chemokine-mediated lymphocyte trafficking during gastric inflammation.

07-03/P

CPG ODN STIMULATES THE MATRIX METALLOPROTEINASE-9 INDUCTION VIA ACTIVATION OF ERK AND P38 MAP KINASE, BUT NOT JNK

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Unmethylated CpG oligodeoxynucleotides (CpG ODNs) can activate immune cells to produce immune mediators. This study demonstrates that CpG ODN-stimulated murine macrophage RAW 264.7 cells produce matrix metalloprotein (MMP)-9. CpG ODN mediated MMP-9 expression is regulated at transcriptional level and requires de novo protein synthesis. Inhibition of extracellular signal-regulated kinase (ERK) and p38 mitogen-activated protein (MAP) kinase, but not Jun N-terminal kinase (JNK), results in severe impairment of CpG ODN-induced MMP-9 expression, although all three kinases could be phosphorylated by CpG ODN. We also observed that CpG ODN elicits NF_B activation and NF-κB is a downstream target of p38 MAP kinase. In summation, our data demonstrate CpG-ODN-triggered MMP-9 expression via ERK, and p38 MAPkinase followed by NF-κB activation.

07-04/P

ESSENTIAL CONTRIBUTION OF A CHEMOKINE, CCL3, AND ITS RECEPTOR, CCR5, TO LUNG METASTASIS

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Alveolar macrophages are one of main cell populations residing in lungs and their numbers are frequently increased under various pathological conditions, including tumor. Accumulating evidence suggests that tumor-associated macrophages can promote tumor progression by producing various growth factors. However, it still remains to be investigated on the regulatory mechanisms of the trafficking of alveolar macrophages and their roles in lung metastasis. Because a chemokine, CCL3, possesses potent chemotactic activity for macrophages, we investigated the effects of the absence of CCL3 and its specific receptor, CCR5, on lung metastasis induced by an intravenous injection of a mouse renal carcinoma cell line, Renca. Multiple macroscopic lung metastasis foci appeared in lungs of wild-type mice at 21 days after an intravenous injection of Renca cells. Concomitantly, CCL3 mRNA expression was enhanced in lungs and CCL3 protein was immunohistochemically detected in tumor cells. Immunohistochemical analysis detected CCR5-positive cells in the periphery of metastasis foci. In contrast, the same treatment caused remarkably lower numbers of metastasis foci in mice deficient in either CCL3 or CCR5, than wild-type mice. Moreover, the numbers of F4/80-positive cells inside metastasis foci, were reduced in CCL3- or CCR5-deificient mice, compared with wild-type mice. Of interest is that intrapulmonary mRNA expression of vascular endothelial growth factor and its receptor, flt-1, was depressed in CCL3- or CCR5-deifient mice, compared with wild-type mice. These observations suggest that the CCL3-CCR5 axis can regulate the intratumoral trafficking of macrophages, a rich source of growth factors and eventually the lung metastasis process.

07-05/P

PRODUCTION AND REGULATION OF EOTAXIN-2/CCL24 IN A DIFFERENTIATED HUMAN CELL LINE, HT93

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Eotaxin plays important roles in accumulation of eosinophils into allergic inflammatory sites. A human leukemic cell line, HT93, when differentiates into eosiophilic lineage incubated with all-trans retinoic acid (ATRA) or ATRA+IL-5, increased expression of CD11b and CCR3 and decreased expression of CD71 as determined by FACS. In addition, they exhibited marked expression of eosinophil-specifice granule proteins, eosinophil peroxidase (EPO) and major basic protein (MBP) by RT-PCR. No eosinophilic differentiation was induced by butyric acid (BA) or DMSO. Marked production of eotaxin-2/CCL24 was observed when the cells were stimulated with ATRA or ATRA+IL-5, but not with BA or DMSO. Neither production of eotaxin-1/CCL11 nor eotaxin-3/CCL26 was observed. Since only 20 to 30% cells become positive for CCR3 incubated with ATRA, we enriched $CCR3^+$ cells by MACS, in order to define the eotaxin-2 producing cells. $CCR3^+$ cells produced marked eotaxin-2 production compared to $CCR3^-$ cells, indicating that differentiated eosinophils are capable of producing eotaxin-2. Since a transcription factor GATA-1 was increased during the ATRA-induced differentiation, we tested the introduction of siRNA against GATA-1, and found that eotaxin-2 production was significantly inhibited. These results indicate that the capacity to produce eotaxin-2/CCL24 is acquired during differentiation into the eosinophilic lineage, which is dependent on GATA-1 expression.

07-06/O

TRANSGENIC MICE REVEAL A CRUCIAL INVOLVEMENT OF CXCR3 AND DIVERGENT ROLES OF ITS LIGANDS IN AN ANIMAL MODEL OF MULTIPLE SCLEROSIS.

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Chemokines and their receptors are intimately involved in the pathogenesis of CNS inflammatory diseases. The chemokine receptor CXCR3 is important for trafficking of activated T-cells and mediates the chemotactic properties of its ligands CXCL9, CXCL10 and CXCL11. Although these chemokines are highly expressed in MS and the corresponding animal model experimental autoimmune encephalomyelitis (EAE), their role in autoimmune diseases of the CNS is not well defined. In order to study the relevance of CXCR3 in autoimmune diseases of the CNS, we examined the course of myelin oligodendrocyte glycoprotein (MOG)-induced EAE over 60 days in CXCR3 gene knockout mice and transgenic mice with astrocyte-restricted expression of the CXCL9 or CXCL10 genes (GF-CXCL9 or GF-CXCL10 mice). Disease induction was comparable in all groups (incidence 100%, average disease onset 9-12d). However, CXCR3 KO and GF-CXCL9 mice had significantly more severe chronic disease. In addition, GF-CXCL9 but not GF-CXCL10 mice had a higher maximal peak score and a higher mortality. A distinct lesion pattern was observed in CXCR3 KO and GF-CXCL9 mice with increased demyelination, axonal degeneration, microglia/macrophage activation and widespread T-cell distribution. FACS analysis revealed an increased number of $CD11b^+$ expressing costimulatory factors in GF-CXCL9 mice, pointing toward a more florid inflammatory process even 60 days after disease onset. We conclude: (1) CXCR3 ligand signalling is not necessary for EAE induction. (2) CXCR3 signalling and perivascular CXCL9 expression act to shepherd T-cells into delimited locations once they have entered the brain. Failure of this process leads to more widespread microglia/macrophage activation and more severe demyelination and axonal damage which impairs recovery. (3) CXCL9 and CXCL10 have non-redundant and functionally complex roles in EAE, which extend beyond T-cell chemoattraction to the brain.

07-07/P

DIFFERENTIAL TEMPORAL AND SPATIAL EXPRESSION OF THE CXCR3 LIGAND GENES IN CENTRAL NERVOUS SYSTEM DISEASE

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Chemokines and their receptors are involved in diverse central nervous system (CNS) insults. The chemokine receptor CXCR3 mediates the chemotactic properties of three related ligands-CXCL9, CXCL10 and CXCL11. In order to investigate whether these three ligands mediate differential roles in the pathogenesis of CNS inflammatory diseases, we examined the temporal and spatial regulation of the CXCR3 ligand genes in the CNS in two different models: myelin oligodendrocyte glycoprotein (MOG)-induced experimental autoimmune encephalomyelitis (EAE), and cerebral malaria (CM) caused by infection with *Plasmodium berghei* ANKA. In both CNS disease models, levels of CXCL9 and CXCL10 mRNA were increased significantly during the acute phase. CXCL11 was also detected during peak disease, albeit at much lower levels. In MOG-EAE, during the recovery phase, expression of CXCL9 and CXCL10 mRNA was reduced, in both the brain and the spinal cord. CXCL11 mRNA was not detectable during recovery in either the spinal cord or the cerebellum. In IFNγ-receptor deficient mice with MOG-EAE, no CXCL9 or CXCL11 mRNA was detectable, while CXCL10 mRNA was significantly reduced. In MOG-EAE, in situ hybridisation analysis revealed CXCL9 RNA in infiltrating T-cells, as well as in a ring of microglia surrounding the leukocytic infiltrates. In contrast, CXCL10 RNA was seen primarily in astrocytes, in a larger ring surrounding the lesions. In CM a similar restricted pattern of CXCL9 and CXCL10 gene expression was observed by microglia and astrocytes, respectively. In addition, strong CXCL9 gene expression was found in the blood vessels. Taken together, these findings highlight the differential temporal and spatial regulation of the CXCR3 chemokine ligand genes in two different models of CNS immunity in which in MOG-EAE at least, IFNγ plays a crucial role. These observations are consistent with the notion that CXCL9 and CXCL10 have differing functions in the pathogenesis of neuroinflammatory disease.

07-08/P

PREDNISOLONE INHIBITS THE RELEASE OF GRANZYME A AND B DURING HUMAN ENDOTOXEMIA

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Inflammation triggers the release of soluble granzymes into the circulation, reflecting cytotoxic activity of natural killer (NK)- and T-cells. Interferon (IFN)-γ-inducible protein-10 (IP-10) and monokine induced by IFN-γ (Mig) are members of the non-ELR CXC chemokine family, which are structurally closely related and share a common receptor, CXCR3, which is only expressed on NK cells and T lymphocytes. Corticosteroids are anti-inflammatory agents of which the *in vivo* effect on cytotoxic NK and T cells are poorly characterized. The aim of the present study was to determine the effects of increasing doses of prednisolone on the release of granzyme A, granzyme B, IP-10 and Mig in healthy humans exposed to lipopolysaccharide (LPS). For this 32 healthy males received prednisolone orally at doses of 0, 3, 10 or 30 mg (N = 8 per group) 2 hours before intravenous injection of *Escherichia coli* LPS (4 ng/kg). All prednisolone doses inhibited the increase of granzyme B levels relative to baseline levels, whereas the highest dose of 30 mg also inhibited granzyme A release. Furthermore, prednisolone caused a dose-dependent inhibition of IP-10 and Mig secretion. In conclusion, this study demonstrates that prednisolone inhibits LPS-induced activation of cytotoxic T and NK cells *in vivo*.

07-09/P

DIFFERENTIAL EXPRESSION OF CHEMOKINES IN VASCULAR SMOOTH MUSCLE CELLS FROM SPONTANEOUSLY HYPERTENSIVE RAT AND NORMOTENSIVE RAT

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The action of chemokines to the vascular inflammation plays a pathogenic role in the development and maintenance of hypertension. In the present study, the expression of chemokine IL-8, MCP-1 and RANTES was investigated in cultured vascular smooth muscle cells (VSMC) obtained from the thoracic aorta of spontaneously hypertensive rat (SHR) and normotensive Wister-Kyoto rat (WKY). The expression of RANTES had no difference between VSMC from SHR and WKY. However, the expressions of IL-8 and MCP-1 mRNA were stronger in VSMC from SHR than in WKY. Expressions of CCR2 and CXCR1 were diminished in VSMC from SHR. We also detected strong expression of IL-8 and MCP-1 in thoracic aortic tissues from SHR, compared to the expression in WKY. The response of VSMC to the dose of LPS on the expression of chemokine genes in SHR was similar to those in WKY. However, the expression of CD14, LPS receptor was diminished in VSMC from SHR. Expressions of LPS-induced IL-8 and MCP-1mRNA were enhanced in VSMC from SHR. A PPAR-γ ligand, 15-deoxy-$\Delta^{12,14}$-prostaglandin J_2 (15d-PGJ_2) which possess anti-inflammatory activity suppressed the expressions of LPS-induced IL-8, MCP-1 and RANTES in VSMC from WKY and LPS-induced MCP-1 and RANTES expressions in SHR. But, the expression of LPS-induced IL-8 mRNA in SHR was increased by 15d-PGJ_2. The expression of PPAR-γ was diminished in VSMC from SHR. Angiotensin II also induced IL-8 and MCP-1 mRNA expressions in VSMC from SHR, but not in WKY. And these inductions of IL-8 and MCP-1 mRNA were decreased by AT_1 receptor antagonist losartan. These results suggest that although MCP-1 has been known to have a role in vascular inflammation and remodeling in Ang II-induced hypertension, IL-8 also has a possibility to play a critical role in the pathogenesis of hypertension in the SHR, and the changes of inflammation-associated receptors in VSMC may be related to the development of hypertension.

07-10/O

ALARMINS ARE NON-COGNATE LIGANDS FOR GαIPCR AND ACTIVATORS OF DENDRITIC CELLS

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Recent studies have identified a group of structurally diverse multifunctional host proteins that are rapidly released following pathogen challenge and/or cell death and, most importantly, are able to both chemotactically recruit and activate dendritic antigen-presenting cells. These potent immunostimulants, including defensins, cathelicidin (LL37) eosinophil-derived neurotoxin (EDN), and high-mobility group box protein 1 (HMGB1), serve as early warning signals to activate innate and adaptive immune systems. They interact with pertussis toxin sensitive chemokine and other receptors on host cells. For example, defensins, LL37, HMGB1 and EDN mimic chemokine and cytokine activities by interacting with CCR6, FPRL-1 and Toll-like receptors (TLR2) respectively. These antimicrobial peptides are constitutively produced and released by leukocytes, keratinocytes and epithelial cells lining the GI, GU and tracheobronchial tree. In addition, they are induced by injurious stimulants and cytokines. These peptides all have in vivo immunoadjuvant effects. We propose to highlight these proteins unique activities by grouping them under the novel term “alarmins”, in recognition of their role in rapidly mobilizing the immune system. (This work is support by the Intramural Program of the NIH, NCI.)

07-11/P

THE DECOY RECEPTOR D6 SCAVENGES INFLAMMATORY CHEMOKINES AT THE FETAL-MATERNAL INTERFACE: A NEW PLACENTAL PROTECTIVE MECHANISM?

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A number of mechanisms operate in placenta to dampen immune reactions, allowing fetal survival. Consistently with this, severe inflammatory conditions in the mother are frequently associated with fetal loss, both in animals and humans. We recently demonstrate that the chemokine non-signalling receptor D6 expressed on endothelial cells lining lymphatic vessels recognizes and target to degradation most inflammatory CC chemokines from the microenvironment, acting as a scavenger receptor. Here we report that D6 is also expressed in placenta, by both the syncytiotrophoblast monolayer, at the very interface between maternal blood and fetus, and invading extravillous trophoblasts. Consistently with this, D6 transcript and protein have been also detected in the choriocarcinoma cell lines JAR and Jeg-3 (representative of invading trophoblast) and BeWo (representative of syncytiotrophoblast). Confocal microscopy analysis in BeWo cells, cultured in order to form a polarized monolayer, demonstrated that this receptor is preferentially expressed on the apical side of the cell. In this cellular model, no evidence for a D6-facilitated transfer of chemokines was observed, while transcytosis of immunoglobulins was readily detectable. Conversely, BeWo cells efficiently scavenge inflammatory CC chemokines, and similar results were obtained with JAR and Jeg-3. The ligand specificity and the lack of expression of signalling CC chemokine receptors are consistent with D6 accounting for observed chemokine scavenging. D6 signalling activity was evaluated in conventional read-outs (calcium fluxes, chemotaxis) in the HTR8 SV40 Neo cell line, representative of extravillous cytotrophoblasts. In CCR5/HTR8 transfectants the D6 and CCR5 ligand CCL3L1/MIP-1αP induced calcium fluxes and cell migration. In the same experimental conditions, D6/HTR8 transfectants did not respond to any of D6 ligand tested, including CCL3L1/MIP-1αP, CCL2/MCP-1, CCL11/eotaxin and CCL22/MDC. In conclusion, D6 is a non-signalling chemokine receptor expressed at the maternal-fetal interface with scavenging activity for inflammatory chemokines and a candidate regulator of inflammation at the fetal-maternal interface.

07-12/O

THE CHEMOKINE DECOY RECEPTOR D6 PROTECTS MICE AGAINST INFLAMMATION- AND AUTOANTIBODY-CAUSED FETAL LOSS

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Chemokines are chemotactic cytokines with a key role in the control of cell trafficking and positioning under homeostatic and inflammatory conditions. D6 is a promiscuous 7-transmembrane-domain receptor which recognizes most inflammatory, but not homeostatic, CC chemokines. It is expressed on lymphatic vessels and in placenta, localized on invading trophoblasts and on the apical side of syncytiotrophoblast cells. Fetal loss in animals and humans is frequently associated with inflammatory conditions, for instance systemic inflammation caused by maternal infection and autoantibodies in the antiphospholipid syndrome cause fetal loss by inducing an inflammatory status and a thrombogenic reaction in placenta. In vitro experiments demonstrated that D6 sustains rapid and efficient ligand internalization and degradation without known signaling. Thus D6 has been defined as a decoy and scavenger receptor for inflammatory CC chemokines. Consistent with this hypothesis, $D6^{-/-}$ mice showed an anticipated and exacerbated inflammatory response in a model of skin inflammation, and the absence of D6 resulted in increase cellularity and inflammatory chemokine levels in draining lymph nodes. Moreover in a model of a systemic inflammation induced by LPS treatment, significantly higher serum concentrations of CCL2/JE, CCL11/Eotaxin, CCL22/MDC, but not CXCL2/MIP-2, were detected in $D6^{-/-}$ mice. Exposure of $D6^{-/-}$ pregnant mice to LPS or antiphospholipid autoantibodies results in increased leukocyte infiltrate in placenta and fetal loss rate, compared with WT mice. Treatement with antibodies blocking inflammatory chemokines prevented fetal damage in $D6^{-/-}$ mice. Thus, D6 is a decoy receptor structurally adapted and strategically located to tune systemic and tissue inflammation.

07-13/O

A PEPTIDE ANTAGONIST OF CXCR4 RESTORES SENSITIVITY OF B16 MURINE MELANOMA CELLS TO KILLING BY GP100-SPECIFIC CTL AND INCREASES THE EFFICACY OF IMMUNE-MODULATING THERAPY FOR ESTABLISHED LUNG METASTASES

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Expression of the chemokine receptor, CXCR4, by tumor cells promotes cancer cell survival and metastasis. Inhibition of CXCR4 with a peptide antagonist, T22, blocks metastatic implantation of CXCR4-transduced B16 (CXCR4-luc-B16) melanoma cells in lung, but not the outgrowth of established metastases, raising the question of how T22 can best be used in a clinical setting. While the treatment of CXCR4-luc-B16 cells in vitro with the CXCR4 ligand, CXCL12, did not reduce killing induced by cisplatin or cyclophosphamide (CPA), CXCL12 markedly reduced Fas-dependent killing by gp100-specific (pmel-1) $CD8^+$ T cells. T22 pretreatment restored sensitivity of CXCR4-luc-B16 cells to pmel-1 killing, even in the presence of CXCL12. Since CPA boosts host anti-tumor immunity in vivo, we used single, low-dose CPA treatment as a model of immunotherapy. Mice inoculated via tail vein with CXCR4-luc-B16 cells on day 0 and treated with T22 on day 4-7 showed equivalent numbers of lung nodules (vs. PBS treatment). While mice treated with CPA (100 mg/kg) on day 5 showed a ~50% reduction in lung nodules compared to PBS treatment, mice treated with T22 (d 4-7) followed by CPA (d 5) showed a further ~70% reduction ($p < 0.05$) in metastases compared to CPA-treated animals. Preliminary experiments also indicated that T22 pretreatment augments the efficacy of anti-CTLA4 mAb treatment of established B16 lung metastases. In summary, CXCR4 activation protected B16 cells from pmel-1 T cell-mediated killing, which was reversed with T22 in vitro. In vivo, T22 alone had no effect on established metastases, but synergized with CPA and anti-CTLA4 mAb treatment in reducing metastatic lesions, suggesting a novel strategy for augmenting the efficacy of immunotherapy.

07-14/P

DIFFERENTIAL EFFECTS OF CYTOKINES ON THE EXPRESSION OF IL-8 AND IP-10 IN HUMAN COLONIC EPITHELIAL CELLS.

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Intestinal epithelial cell functions may be influenced on one side by agents coming from the lumen and on the other side by cytokine producing immune cells. It is known that in the mucosa of patients with inflammatory bowel disease (IBD) IL-8 and IP-10 expression is increased. Epithelial cells are thought to play a role in signaling the influx of leukocytes during the acute and chronic mucosal inflammation. To further explore this role, the regulation of IL-8 and IP-10 gene expression by cytokines was characterized in human intestinal epithelial cell lines Caco-2, HT29 and DLD1. IL-8 and IP-10 synthesis and secretion kinetics were assessed by Real-Time PCR, northern blotting, enzyme-linked immunoabsorbent assay and transient transfections in Caco-2, HT-29 and DLD1 cells, treated with IL-1β, TNFα, IFNγ alone or in combination with each other. The induction of IL-8 and IP-10 mRNA in all three cell lines started very early after stimulation with IL-1β and TNFα. IP-10 mRNA induction by IFNγ alone was delayed and reached a peak at 8hr. IFNγ had no effect on IL-8 induction in all three cell lines. However, IFNγ in combination with TNFα showed a synergistic effect on the induction of IL-8 in HT29 cells. IL-1β and TNFα strongly enhanced the effect of IFNγ on IP-10 expression in all three cell lines. Chemokine mRNA induction was accompanied by an increase of chemokine protein in cell culture supernatants. IFNγ led to a weaker induction of the IP-10 promoter compared with IL-1β in Caco-2. Our results stress the importance of intestinal epithelium being not solely a pure barrier against intestinal antigens but being actively involved in the recruitment of inflammatory cells. The synergistic actions of proinflammatory cytokines on chemokine expression reflects the redundancy of the recruitment process, which should be taken into account when treating IBD.

07-15/P

THE ROLE OF ONCOSTATIN M IN CHEMOKINE REGULATION

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Oncostatin M (OSM) is a pleiotropic cytokine of the IL-6 family that is secreted by activated macrophages, T lymphocytes and neutrophils. OSM and IL-6 have previously been shown to regulate the expression of various chemokines and a series of inflammatory mediators in human peritoneal mesothelial cells (HPMC) (Hurst et al., 2001; 2002). To delineate the potential involvement of OSM verse IL-6, initial studies have examined the control of chemokine responses in OSM stimulated HPMC. OSM signalling in humans is governed by a receptor complex consisting of gp130, which acts as the universal signalling receptor for all IL-6-related cytokines, and either the leukaemia inhibitory factor receptor (LIFR) or the OSM specific receptor (OSMRβ). Furthermore, OSMRβ can also interact with an IL-31 binding subunit (IL-31Rα) to form a selective IL-31 receptor complex. Flow cytometric analysis of primary HPMC isolates showed that these cells express gp130 and OSMRβ, but not LIFR, whilst initial RT-PCR studies also confirmed a lack of IL-31Rα expression. To assess chemokine activation by OSM, HPMC were stimulated with OSM (0.03-30ng/ml) and levels of CCL2, CCL5, CXCL8, CXCL9, CXCL10 and CXCL11 were measured using ELISA. OSM induced release of CCL2 and CXCL10 in a dose-dependent manner. Treatment of HPMC with IL-1 and OSM synergistically increased production of CCL2, however OSM significantly reduced IL-1 stimulated induction of CXCL8. OSM was also shown to significantly decrease γ-IFN stimulated induction of CXCL9, CXCL10 and CXCL11. To investigate whether stimulation with OSM had any effect on the expression of OSMRβ on HPMC, FACS analysis was performed on cells stimulated with OSM (30ng/ml). There was no significant change in expression after treatment with OSM, IL-1 or γ-IFN. These data confirm that OSM signalling through a gp130:OSMRβ receptor complex has the potential to differentially promote the expression of inflammatory chemokines that may ultimately influence the

profile of leukocyte trafficking observed during an inflammatory response. Subsequent studies using a model of peritoneal inflammation in OSMRβ-deficient mice are currently being used to evaluate the importance of this activity.

07-16/P

IDENTIFICATION OF CXCL11 AS A STAT3-DEPENDENT GENE INDUCED BY IFN.

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IFNs are multifunctional cytokines that selectively regulate gene expression through several signaling pathways. The present study explored the involvement of STAT3 in the IFN-induced expression of the gene encoding the CXCL11 chemokine also called βR-1 and I-TAC. The CXCL11 gene was induced in IFN-sensitive Daudi cells, but not in an IFN-resistant DRST3 subline with a defective STAT3 signaling pathway. Expression of wild-type STAT3 in DRST3 cells restored the IFN inducibility of CXCL11. In contrast, the IFN-induced gene ISG15 was induced to a similar extent in both Daudi and DRST3 cells. Chromatin immunoprecipitation (ChIP) assays demonstrated that IFN treatment of Daudi and DRST3 cells induced the binding to the CXCL11 promoter of STAT3, which was phosphorylated on Tyr-705 (canonical STAT dimerization site). Reconstitution of STAT3 knockout mouse embryonic fibroblasts with wild-type-STAT3 or STAT3 with Tyr-705 mutated restored IFN inducibility of the CXCL11 gene. These data indicate that CXCL11 gene induction by IFN is STAT3-dependent, but that phosphorylation of Tyr-705 of STAT3 is not required. ChIP assays also revealed that NF-κB family member p65 and interferon regulatory factor 1 (IRF1) were bound to CXCL11 promoter upon IFN treatment of Daudi cells. In contrast, IFN induced the binding of p50 and IRF2 to the CXCL11 promoter in DRST3 cells. Thus, the recruitment of the transcriptional activators p65 and IRF1, and the displacement of the transcriptional repressors p50 and IRF2 from the CXCL11 promoter also appear to regulate the IFN induction of CXCL11 gene transcription.

07-17/O

IMPACT OF THE CHEMOKINE INTERCEPTORS DARC AND D6 ON CHEMOKINE HOMEOSTASIS

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Chemokines are homologous secreted proteins, which exert their biological effects through binding to multiple specific G-protein coupled receptors with seven transmembrane domains. In addition to classical signaling receptors, chemokines bind to silent receptors, so called interceptors. These serpentine receptors are not coupled to G-proteins and subsequent signaling cascades. Nevertheless, interceptors may influence chemokine homeostasis by either transporting chemokines across biological barriers or by their degradation. In this study we investigated the role of interceptors DARC and D6 in i.) binding, ii.) degradation and iii.) unidirectional transport of chemokine CCL2 (MCP-1) using transfected cell lines and transwell system. In addition, we determined how these functions impact on the capacity of DARC and D6 to support monocyte migration across the cell monolayers. Furthermore, in order to mimic the blood recirculation we repeatedly exchanged the fluid in the upper chamber. This procedure allowed us to differentiate between the migratory effect of soluble and cell surface-immobilized CCL2. We detected an increased binding of CCL2 to cells transfected with DARC and D6, compared to the control cells. DARC transfectants transported CCL2 from basolateral to apical side only, but not vice versa, and did not degrade this chemokine in the process. D6 transfectants efficiently degraded CCL2. This is in agreement with the decoy function described for this interceptor. In addition, D6 transported intact chemokine across the monolayers in both directions. The expression of DARC by the monolayer increased several fold the number of monocytes migrated to CCL2. Conversely, the expression of D6 had little impact on monocytes transmigration. Our results clearly demonstrate that interceptors significantly influence the biology of chemokines. This should be considered when investigating chemokine function and designing therapeutics targeting chemokines and their receptors. Also, interceptors themselves may become attractive therapeutic targets in inflammatory diseases.

07-18/O

ADIPOSE TISSUE TRANSPLANTATION RESTORES THE INFLAMMATORY RESPONSE TO DEXTRAN SULFATE SODIUM IN LEPTIN-DEFICIENT MICE

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Leptin-deficient *ob/ob* mice are characterized by markedly reduced inflammatory responses, particularly in the gastrointestinal tract. Administration of recombinant leptin reverses these defects. However, there is still controversy as to whether systemic leptin derived from adipose tissue or leptin that is locally produced in the intestine is important in regulating colonic inflammation. Our aim was to evaluate whether transplantation of WT adipose tissue into *ob/ob* mice is able to mimic the effect of recombinant leptin administration. A group of 10 female *ob/ob* mice (C57BL6, 6 –week-old) received subcutaneous transplantation of approximately 1 g of adipose tissue obtained from WT C57BL6 females. A separate group of *ob/ob* mice was sham-operated. Eight weeks after transplantation, serum leptin levels were 0.6 +/- 0.1 ng/ml in the *ob/ob* transplanted group compared to 4.1 +/- 1.0 ng/ml in WT mice. As expected, serum leptin was below detection limit in non-transplanted *ob/ob* mice. Despite the fact that adipose tissue transplantation raised leptin levels to only 15% of those observed in WT mice, this was sufficient to normalize metabolic abnormalities (glycemia, ALT, liver weight) in *ob/ob* mice and to stop body weight gain. Transplanted and non-transplanted *ob/ob* mice differed in their responsivity in the model of DSS-induced colitis. As previously reported, non-transplanted *ob/ob* mice developed only minor colonic inflammation in response to DSS. Adipose tissue transplantation restored the response to levels equivalent to those of WT mice. Colonic cytokine production was markedly reduced in the non-transplanted *ob/ob* group compared to transplanted *ob/ob* and WT mice. Our data indicate that adipose tissue transplantation is an effective way to restore immune and inflammatory responses in *ob/ob* mice. Adipose tissue-derived leptin is sufficient to normalize responsivity to DSS even in the absence of locally produced leptin. Furthermore, the threshold of leptin necessary to normalize immune and inflammatory function is lower than levels present in lean WT mice.

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07-19/O

CCR7 IS REQUIRED FOR THE *IN VIVO* FUNCTION OF CD4+ CD25+ REGULATORY T CELLS

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CCR7-mediated migration of naïve T cells into the secondary lymphoid organs is a prerequisite for their encounter with mature dendritic cells, productive presentation of cognate antigen and consequent T cell proliferation and effector differentiation. Therefore, it has been widely believed that CCR7 is required for the initiation of adaptive immune responses. In contrast, we show that primary immunity can develop in the absence of CCR7. Moreover, CCR7 deficient mice display enhanced immune responses to exogenous antigens. Our data cumulatively suggests that enhanced immunity in CCR7 deficient mice is due to defective lymph node (LN) positioning of CD4+ CD25+

regulatory T cells (Tregs) and consequent impediment of their function. The FoxP3+ Tregs express CCR7 and following their adoptive transfer migrate into the LN of wild type mice. Here they proliferate *in situ* upon antigen stimulation and inhibit the generation of antigen-specific T cells. Conversely, transferred CCR7 deficient Tregs fail to migrate into the LN and suppress antigen-induced T cell activation. Transfer of combinations of naïve and Treg cells from wild type and CCR7 deficient mice into syngeneic SCID mice demonstrates that CCR7 deficient Treg cells, unlike their wild type counterparts, cannot prevent the development of inflammatory bowel disease.

07-20/P

THE PF-4 VARIANT CXCL4L1 IS A POTENT INHIBITOR OF ANGIOGENESIS AND TUMOR GROWTH

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Blood platelets are activated by chemokines and are important components of hemostasis, contribute to wound healing by forming thrombi and initiate repair processes. In addition, chemokines influence tumor growth by regulating angiogenesis. Platelet factor-4 (PF-4)/CXCL4 was the first chemokine described to inhibit neovascularization. Recently, the product of the non-allelic variant gene of CXCL4, PF-4_{var1}/PF-4_{alt}, designated CXCL4L1 was isolated from thrombin-stimulated human platelets and purified to homogeneity. Although secreted CXCL4 and CXCL4L1 differ in only 3 amino acids, CXCL4L1 bound heparin with lower affinity than CXCL4 but was more potent in inhibiting chemotaxis of human microvascular endothelial cells (HMVEC) toward interleukin-8 (IL-8/CXCL8) or basic fibroblast growth factor (bFGF). *In vivo*, CXCL4L1 was also more effective than CXCL4 in inhibiting bFGF-induced angiogenesis in rat corneas. Human CXCL4L1 cDNA was cloned and expressed in *E.coli*. Both *in vitro* and *in vivo* angiostatic activities were confirmed with recombinant CXCL4L1. Furthermore, the chemotactic activity of CXCL4L1 for various leukocyte subtypes was compared with that of authentic CXCL4 and other chemokines. Finally, the antitumor activity of CXCL4L1 was evaluated in parallel with CXCL4 using the mouse B16 melanoma model. It was found that CXCL4L1 injected intratumorally significantly reduced tumor growth with a lower minimal effective dose than CXCL4. Thus, activated platelets release CXCL4L1, a potent regulator of endothelial cell biology, which affects vascular diseases and tumor growth.

07-21/P

REGULATION OF DOPAMINERGIC NEURON DEVELOPMENT BY ALFA-CHEMOKINES

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Chemokines and their receptors were initially recognized as playing a role in leukocyte migration and communication. These molecules control immune cell trafficking and recirculation of the leukocyte population between the blood vessels, lymph, lymphoid tissues and organs. During the past years, a growing body of evidence suggests that chemokines and their receptors also mediate intercellular communication in the central nervous system. It has previously been shown that members of the alfa-chemokine family contribute to the mechanisms that control proliferation, migration, and maturation of several subtypes of precursors during development of different areas of the central nervous system. For example, CXCL1/CXCR2 signaling is involved in patterning of the spinal cord by controlling the positioning of oligodendrocyte precursors. Also, the CXC ligand CXCL12 and its cognate receptor CXCR4 are required for normal development of the hippocampal dental gyrus and are implicated in proliferation of cerebellar granule cell precursors in cooperation with Sonic hedgehog. In this study, we asked the question whether the two alfa-chemokines, CXCL6 and CXCL8, also play a role in development. We found that alfa-chemokine receptor CXCR2 is expressed in precursor populations in the ventral midbrain (VM). This receptor binds to both CXCL6 and CXCL8. When treating cells with CXCL6 and CXCL8 we detected that both chemokines promoted the differentiation of Nurr1+ precursors into dopaminergic neurons in vitro, but only CXCL8 enhanced their proliferation. The finding that CXCL8 regulated both proliferation and differentiation of VM precursors prompted us to examine whether CXCL8 could be applied to expand and differentiate VM neurospheres. Our results show that this is the case and suggest that alfa-chemokines may find an application in cell replacement therapy for Parkinson's diseases, as a tool to enhance the number of dopaminergic neurons in precursor/stem cell preparations.

07-22/P

FUNCTIONAL CXCR4-EXPRESSING MICROPARTICLES ARE ASSOCIATED WITH PATHOGENESIS OF ACUTE MYELOID LEUKEMIA

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Stromal cell-derived factor-1 (SDF-1/CXCL12) and its receptor CXCR4 are implicated in a variety of human physiological and pathological conditions including acute myeloid leukemia (AML). Cellular microparticles (MPs) are also associated with human pathology. In the present study we examined the putative relationships between CXCR4/SDF-1 axis and MPs in AML. Via combination of flow cytometry, ELISA and Western blot, we detected CXCR4-expressing MPs ($CXCR4^+$MPs) in the peripheral blood (PB) and bone marrow (BM) plasma samples of normal and newly diagnosed adult AML patients. In AML patients, the levels of $CXCR4^+$MPs and SDF-1 in the PB and BM plasma samples were elevated as compared to normal individuals. Remarkably, we found strong correlations between the levels of $CXCR4^+$MPs and intact (non-cleaved) SDF-1 in the PB and BM plasma, and white blood cell count. Our data indicate also N-terminal truncation of CXCR4 molecule in the MPs isolated from AML patients. Despite this, $CXCR4^+$MPs were capable to transfer the CXCR4 molecule to AML-derived HL-60 cell line, enhance their migration to SDF-1 in vitro and increase their homing to the BM and spleen of sublethally irradiated NOD/SCID mice. The CXCR4 antagonist AMD3100 attenuated these effects. These findings suggest that functional $CXCR4^+$MPs and SDF-1 are involved in AML pathogenesis and progression. Their levels are potentially valuable as the additional diagnostic AML parameter.