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Israel Immunological Society

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**ספר ההרצאות והתקצירים**  
**Lectures and Abstracts Book**

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# **Invited Speakers**

**1 – 18**

# Gene Regulation in Melanoma Progression: Application for Bioimmunotherapy

Bar-Eli Menashe, Department of Cancer Biology, University of Texas M. D. Anderson Cancer Center

The molecular changes associated with the transition of melanoma cells from radial growth phase (RGP) to vertical growth phase (VGP, metastatic phenotype) are not yet well defined. We have demonstrated that the progression of human melanoma is associated with loss of expression of the transcription factor AP-2. In metastatic melanoma cells, this loss resulted in overexpression of MCAM/MUC18 and MMP-2 and lack of c-KIT expression. In addition, inactivation of AP-2 in primary cutaneous melanoma cells by dominant-negative AP-2 (AP-2B) augmented their tumorigenicity in nude mice. We have also recently demonstrated that loss of AP-2 expression in metastatic melanoma cells resulted in over production of the thrombin receptor, PAR-1, which in turn, contributes to the metastatic phenotype of melanoma by upregulating the expression of adhesion molecules, proteases and angiogenic factors, such as IL-8. Based on these observations, we have developed two fully humanized antibodies in an attempt to inhibit angiogenesis, tumor growth and metastasis of human melanoma. One antibody (ABX-MA1) is directed against the adhesion molecule MCAM/MUC18 while the second (ABX-IL8) is a neutralizing antibody for IL-8. In preclinical studies these antibodies when used as a single modality were shown to inhibit the growth, angiogenesis and metastasis of highly metastatic cells in nude mice. The possibility of using these antibodies in combination with chemotherapy to treat melanoma patients will be discussed.

# The chemokines CCL5 and CCL2 in breast cancer: regulation, pro-malignancy activities and prognostic implications

Ben-Baruch, A.<sup>1</sup>, E. Azenshtein<sup>1\*</sup>, G. Soria<sup>1\*</sup>, S. Shina<sup>1</sup>, N. Barak<sup>2</sup>, L. Trejo-Leider<sup>3</sup>, K. Haim<sup>1</sup>, T. Meshel<sup>1</sup>, E. Neumark<sup>1</sup>, I.P. Witz<sup>1</sup>, I. Keydar<sup>1</sup>

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The chemokines CCL5 and CCL2 are important potential contributors to breast malignancy, e.g. by virtue of their ability to promote the presence of deleterious macrophages at tumor sites, and to increase vascularization and angiogenicity. The expression of CCL5 and CCL2 is almost totally confined to the tumor cells as compared to adjacent normal duct cells, suggesting that the expression of the two chemokines is associated with transformation events taking place in breast epithelial cells. Moreover, the expression of CCL5 and CCL2 is associated with advanced disease and early relapse, and our studies indicate that CCL5 expression is a significant predictor of disease progression in stage II breast cancer patients. Further, our findings suggest that it is not one of the chemokines, but actually a combined expression of both, which drives forward the progression of breast cancer. Accordingly, an intensive cross-talk exists between CCL5 and CCL2 in breast tumor cells, evidenced by the ability of CCL2 to potently increase the expression of CCL5 by breast tumor cells. These effects of CCL2 do not result from increased transcription or translation of CCL5, rather CCL2 may prevent CCL5 intracellular degradation, followed by release of excessive CCL5 by the cells. The process of CCL2-induced CCL5 up-regulation in breast tumor cells is mediated by a variety of signaling pathways, including Erk and PI3K activation. Overall, our studies suggest that CCL2 acts in situ to amplify pro-malignancy cascades by elevating CCL5 secretion by breast tumor cells, thus supporting breast malignancy.

## **Tissue specific control of NF–kappaB activation**

Ben–Beriah Yinon, Lee J, Horwitz E, Cojocaru G, Kanarik N, Alkalay I, Eckmann L, Pikarsky E Raz E.

University of California San Diego and Hebrew University–Hadassah Medical School

Canonical NF–kappaB activation targets IkappaB and p105/NF–kappaB1 to degradation via the ubiquitin proteasome system. We and others have previously shown that beta–TrCP is a key regulator in this system; its two isoforms beta–TrCP1 and 2 are both necessary and sufficient to control IkappaB stability. Ongoing experiments in our lab attempt to unveil the unique functions of each beta–TrCP isoform in different tissues and determine the consequence of specific isoform inhibition. So far, beta–TrCP–controlled degradation has been mainly studied in standard tissue culture conditions, yet many tissues and particularly epithelial cells are configured in vivo in a specific orientation. We will describe a cellular mechanism whereby the polarity of gut epithelium dictates the outcome of NF–kappaB signaling, thereby playing a major role in colonic homeostasis. TLR9 activation via apical and basolateral surface domains have distinct transcriptional responses. Whereas basolateral TLR9 signals NF–kappaB activation, apical TLR9 invokes intracellular tolerance against subsequent basolateral TLR9 and other TLRs' challenges. TLR9–deficient mice lacking the tolerizing receptor are triggered much more rapidly for NF–kappaB activation and are highly susceptible to experimental colitis. Our data provide a case for an organ–specific innate immunity where TLR regulation evolved to maintain colonic homeostasis. This is achieved by a novel cellular mechanism, where the different surface domains of a polarized cell dictate an opposing signaling outcome to a similar stimulus.

# Epigenetic regulation of V(D)J recombination and somatic hypermutation

Bergman Yehudit, The Hebrew University Medical School

The process called variable (diversity) joining V(D)J recombination is controlled at three basic levels: lineage specificity, temporal order in the lineage, and allelic exclusion. An increasing body of evidence indicates that many epigenetic mechanisms are involved in the regulation of V(D)J recombination. We have focused on the activation of Igk during B cell development. Our findings indicate that this locus undergoes a programmed series of epigenetic changes that take place in a stepwise way, ultimately rendering one Igk allele preferentially accessible to rearrangement. These events proceed 'outside-in', first at the level of nuclear organization, then at the level of chromatin (histone modification) and finally at the level of DNA (demethylation). Moreover, our experiments suggest that allelic exclusion is mediated by a predetermined "instructive" mechanism that begins early in development when the Igk alleles first become asynchronously replicated. Once established, this differential state is then maintained in a clonal way in somatic cells, but only during pre-B cell development is this signal used to direct subsequent epigenetic modifications that possibly mark one allele as a better substrate for the recombination machinery. Furthermore, we show that the epigenetic mechanisms that initially bring about monoallelic V(D)J rearrangement continue to play a role in directing allele specificity for somatic hypermutation, thus controlling antibody diversity at later stages of B-cell development.

# **Sculpting the Gut by Developmental Signaling Pathways: LKB, Peutz–jehhers syndrome and cell polarity**

Clevers Hans

To be announced

# The Immunological Homunculus

Irur R. Cohen, Yifat Merbl, Merav Toledano and Francisco Quintana,  
The Weizmann Institute of Science

The Immunological Homunculus (IH) refers to the immune system's internal image of key molecules in the body. The natural autoimmune repertoires of T cells and B cells are important components of the IH. Here we used an antigen microarray device (antigen chip) to analyze informatically (with clustering algorithms, and correlation mapping) the natural IgM, IgA and IgG autoantibody repertoires present in 10 pairs of sera from healthy mothers

and their newborn babies, binding to 305 different molecules – mostly self–molecules. Unlike IgG antibodies, IgM and IgA antibodies do not cross the placenta from mother to fetus, and so IgM or IgA autoantibodies in cord serum must originate from prenatal immune activity in the developing baby.

We now report that different babies manifest cord IgM autoantibodies to a relatively uniform set of self–molecules – the primordial IH; these prenatal IgM autoantibodies are highly correlated among different babies, and the cord IgM autoantibodies as a group cluster separately from maternal IgM autoantibodies. Cord IgA autoantibodies are less uniform among different

babies than are cord IgM autoantibodies, but still can be clustered separately from maternal IgA autoantibodies. In contrast to cord IgM and IgA, the natural IgG autoantibodies of cord and mother are identical. Thus, IH natural autoimmunity begins in utero in healthy humans. Unexpectedly, many cord autoantibodies bind self–molecules associated with major autoimmune diseases.

The relative rarity of clinical autoimmune disease, however, implies that congenital IH autoantibodies are benign, if not advantageous for body maintenance.

## **Peripheral B cell receptor editing may promote the production of high affinity autoantibodies in CD22-deficient mice**

Eilat Dan, Fischel R., Kat E., Yachimovich-Cohen N. Yarkoni Y., Hadassah University Hospital and Hebrew University Faculty of Medicine

CD22-deficient mice are characterized by B cell hyperactivity and autoimmunity. We have constructed knock-in CD22-deficient mice, expressing an anti-DNA heavy (H) chain (D42), alone or combined with Vk1-Jk1 or Vk8-Jk5 light (L) chains. The Ig-targeted mice produced a lupus-like serology, that was age and sex dependent. High affinity IgG autoantibodies were largely dependent on the selection of B cells with a particular H/L combination, in which a non-transgenic, endogenous L chain was assembled by secondary rearrangements through the mechanism of receptor editing. Moreover, we present evidence that these secondary rearrangements are very prominent in splenic peripheral B cells. Since CD22 is primarily expressed on the surface of peripheral B cells, we propose a model for the development of a lupus-like autoimmune disease by a combination of peripheral receptor editing and abnormal B cell activation.

## **Is PICOT a putative functional linker between PKC $\zeta$ and EED in TCR-induced transcriptional regulation of T Lymphocytes?**

Isakov Noah, Department of Microbiology and Immunology and the Cancer Research Center, Ben Gurion University of the Negev

The critical role of PKC $\zeta$  in T cell activation is now well established, but its mode of regulation during the activation response has not been fully defined. In an attempt to identify putative regulators of PKC $\zeta$ , we performed a yeast two-hybrid screen of a human T cell cDNA library, and discovered a novel protein, termed PICOT, which specifically interacts with PKC $\zeta$  and regulates PKC $\zeta$ -dependent functions in activated T cells. Human PICOT possesses an N-terminal thioredoxin-like homology domain, followed by a tandem repeat of a unique sequence, termed PICOT homology domain (PICOT-HD), but the biological functions of PICOT or its PICOT-HD are unknown.

Our preliminary data suggest that PICOT possesses a functional nuclear export signal sequence and that it can undergo tyrosine phosphorylation and translocation to the nucleus of activated T cells. PICOT expression increases in-vitro in mitogen triggered proliferating T cells, and in-vivo in anaplastic large cell lymphoma (ALCL) and Reed-Sternberg cells of Hodgkin's lymphoma. In addition, using its PICOT-HD, it can mediate direct physical interaction with the protein product of the Polycomb-group gene embryonic ectoderm development (eed), a regulator of thymocyte differentiation and a suppressor of thymic lymphoma development. Our current studies aimed at clarifying whether PICOT is involved in the regulation of T cell proliferation by linking TCR-proximal events that result in the activation of PKC $\zeta$  to remote activation events that affect transcriptional regulators, such as EED, and thereby regulating the transcription of critical growth promoting genes.

# Innate and Adaptive Immune Functions of Dendritic cells

Jung Steffen, Weizmann Institute of Science

Dendritic cells (DC) are specialized migratory mononuclear phagocytes (MP) that are believed to have co-evolved with the acquired immune system. They are unrivaled in their potency to activate naïve T cells and ensure efficient immuno-stimulation. Paradoxically, however, DC, are also critical regulatory cells that curb the inherent auto-reactivity of the immune system. Both of these activities rely on the cognate interaction of antigen-specific T cells and antigen presenting DC, but it remains unknown how DC can fulfill these seemingly opposing tasks. The answer to this puzzle could lie in DC heterogeneity and the existence of multiple DC subsets. However, while a number of recent studies support the interesting scenario that DC subsets play different roles in innate immune defense, as well as T cell tolerization and stimulation, solid experimental evidence for task division within the DC compartment remains rare. Here we will report on our progress in using conditional in vivo DC ablation to define physiological functions of DC and DC subsets. If DC subsets have differential indeed in vivo functions, manipulation of DC compartments could have therapeutic value. Such manipulations will however require an in depth understanding of DC in vivo origins. We have therefore begun to investigate the in vivo differentiation potential of adoptively transferred DC precursor grafts. The recent characterization of a novel DC and Macrophage committed BM precursor (MDP) (Fogg et al. Science 2005) allowed us to establish a complete sequence of myeloid differentiation from the BM-resident MDP to the peripheral DC.

## **IRF-8 is obligatory for the expression of PML tumor suppressor gene in myeloid cells**

Levi Ben-Zion, Dror Noy, The Department of Biotechnology & Food Engineering, Technion

Interferon (IFN) Regulatory Factor-8 (IRF-8) and IRF-1 are myeloid cell essential transcription factors that belong to the IRF family and exert some of their effect through the formation of transcriptional heterocomplexes. Interestingly, mice with null mutation in IRF-8 are defective in the ability of myeloid progenitor cells to mature towards macrophage lineage whereas mice with null mutation of IRF-1 are defective in the differentiation to granulocytes. Accordingly, IRF-8<sup>-/-</sup> develop Chronic Myelogenous-like Leukemia (CML). In humans, IRF-8 expression is reduced in CML patients and recovers to normal levels during treatment-mediated remission. In addition, low expression of IRF-1 and aberrantly-spliced forms of IRF-1 were reported in patients with CML. Together, these observations point to the role of IRF-8 and IRF-1 as CML tumor suppressor genes. Using DNA microarray, we have searched for macrophage specific target genes regulated by these two transcription factors. Our results demonstrate that the Promyelocytic Leukemia (PML) gene is regulated by both IRF-8 and IRF-1 in macrophages. PML is a tumor suppressor gene that serves as a scaffold protein for nuclear bodies (NBs). We show that in macrophages, a specific isoform(s) of PML is regulated by these two transcription factors. In addition, myeloid cells from IRF-8<sup>-/-</sup> are devoid of PML-NBs that are reformed only upon transduction of IRF-8. Together, these results point to the pivotal role of IRF-8 in myeloid cell differentiation and the modulation PML-NBs. Since IRF-8 is associated with CML and regulates PML, we search for correlation between PML levels and the progression of CML. The levels of both IRF-8 and PML were significantly lower in CML patients than in healthy donors. These data point to a novel link between dysregulated expression of PML and CML.

## **A novel synthetic peptide for the specific treatment of lupus: Mechanisms of action.**

Edna Mozes.<sup>1</sup>, Amir Sharabi.<sup>1</sup>, Heidy Zinger.<sup>1</sup>, Molly Dayan.<sup>1</sup> and Uri Sela.<sup>1</sup>

<sup>1</sup>Department of Immunology, The Weizmann Institute of Science, Rehovot 76100, Israel.

Systemic lupus erythematosus (SLE) is an autoimmune disease characterized by autoantibodies and systemic clinical manifestations. Treatment with a peptide designated hCDR1, based on the complementarity determining region 1 of an anti-DNA autoantibody, ameliorated the serological and clinical manifestations of either spontaneous or induced SLE in mice. The beneficial effects of hCDR1 were associated with a decreased secretion and expression of the pathogenic cytokines, IFN-gamma, IL-10, IL-1beta and TNF-alpha and with an up-regulation of the immunosuppressive cytokine, TGF-beta.

Treatment with hCDR1 up-regulated the CD4+CD25+CD45RB(low) cells expressing Foxp3, CTLA-4 and TGF-b. Adoptive transfer of hCDR1-treated splenocytes to SLE afflicted mice down-regulated all disease manifestations. Depletion of the CD25 expressing cells diminished significantly the therapeutic effects of hCDR1, whereas administration of the enriched CD4+CD25+ cell population was beneficial to the diseased mice. The hCDR1-induced regulatory cells suppressed the activation of autoreactive CD4+ cells as indicated by the diminished expression of CD69 and Fas ligand on the latter, leading to reduced rates of apoptosis. Treatment with hCDR1 down-regulated T cell adhesion and chemotaxis via the up-regulated TGF-beta. The latter was accompanied by the diminished ERK phosphorylation (known to participate in the signalling cascade that is involved in cell locomotion). Further, hCDR1 induced suppression of ERK-phosphorylation resulted in the diminished expression and function of a pair of key cell adhesion receptors, LFA-1 and CD44, which operate as accessory molecules in mediating APC-T-cell interactions. Finally, hCDR1 was shown to inhibit TCR signalling (ZAP-70 phosphorylation) and to up-regulate mRNA expression of two negative regulators of TCR activation, namely Foxj1 and Foxo3a. Thus, treatment with hCDR1 leads to a cascade of events that culminates in the down-regulation of SLE-associated autoreactive responses and in the clinical improvement of experimental SLE.

## **The involvement of CD44, a molecule with 1000 different faces, in Cancer and autoimmune diseases**

David Naor.<sup>1</sup>, Shlomo Nedvetzki.<sup>1</sup>, Nathalie Assayag.<sup>1</sup>, Shulamit B.<sup>1</sup> Wallach–Dayan.<sup>1</sup> and Itshak Golan.<sup>1</sup> <sup>1</sup>The Lautenberg Center for General and Tumor Immunology, the Hebrew University– Hadassah Medical School, Jerusalem, Israel

Alternative splicing can theoretically generate close to 1000 different CD44 isoforms by differential utilization of 10 variant exons. At present, several dozen of CD44 variants have been already discovered. Both normal cells and cells engaged in pathological activities use cell surface CD44 for multiple functions, including support of cell migration and delivery of apoptotic signals. We predicted that the expression of distinct CD44 isoforms on Normal cells, cancer cells and cells involved inflammatory autoimmune diseases may allow the production of disease–specific monoclonal antibodies against disease–related CD44 variants, expressed exclusively on the pathological cells. This prediction has been challenge by an experimental approach, which will be reported in the 2006 meeting of the Israel Immunology Society.

## **Pattern recognition by NK cells and involvement of DC**

Porgador Angel, Department of Microbiology and Immunology and the Cancer Research Center, Ben Gurion University of the Negev.

The hallmark of the innate immune system is the recognition of pathogen-associated molecular patterns (PAMPs) by a limited number of germline-encoded receptors. Natural killer (NK) cells constitute a highly specialized lymphoid population functionally identified by its potent cytolytic activity against foreign, tumor and virus-infected cells. Being a fundamental component of the innate immune system, the NK cell recognizes and is triggered by molecular patterns, such as those of transformed or virus-infected cells. Pattern recognition by NK cells is not restricted to PAMPs; rather NK cells may be triggered by molecular patterns that are not exclusive to microbes, e.g. the missing self trigger and stress-induced pattern. We studied pattern recognition by natural cytotoxicity receptors (NCRs); NCRs are expressed by NK and trigger NK lysis of tumor and virus-infected cells upon interaction with cell-surface ligands of these target cells. Our results describing the patterns recognized by those NCRs and recognition sites on NCRs will be presented. Pattern recognition could be the basis also for NK-dendritic cells (DCs) interactions; several studies have demonstrated that reciprocal activations ensue upon NK/DC interactions. We studied these interactions in IL-1-deficient mice. NK activation due to IL-1beta deficiency and the involvement of DC in this process will be presented.

## Dendritic cells at the interface between innate and adaptive immunity

P. Ricciardi–Castagnoli, M. Foti, , F. Granucci,  
Dept. of Biotechnology and Bioscience, University of Milano–Bicocca, Milano, Italy

Immunity is the result of co–evolution of the immune system and microbes. In the immune system, dendritic cells (DC) act as sentinels for incoming antigens and participate to the innate response. Nevertheless, they are also able to prime adaptive immunity. The strategy of DCs to accomplish these biological effects resides in their ability to segregate in time different functions starting from the pathogen arrival. They respond, in a few hours, to infectious agents by recognizing molecular patterns typical of micro–organisms and absent in self–tissues; they mount a late response that discriminates among different microbes giving rise to memory and, finally, they maintain tolerance against self–proteins.

We have used a genome–wide approach to study how DCs interact with live pathogens during an in vitro infection to reveal the kinetic of differentially expressed genes. The most unanticipated finding of global gene expression analysis on maturing myeloid DCs was that they produce IL–2 transiently at early time points following activation with bacteria, but also parasites and helminth. An inducible type I IFN–dependent pathway leading to an inflammatory signature was also shown in myeloid DC treated with *Schistosoma* eggs showing that DC, depending on the type of environmental signal they encounter, acquires specific DC immune functions.

Granucci F, Foti M, Ricciardi–Castagnoli P. Dendritic cell biology. *Adv Immunol.* 2005;88:193–233;

Feau S, Facchinetti V, Granucci F, Citterio S, Jarrossay D, Seresini S, Protti MP, Lanzavecchia A, Ricciardi–Castagnoli P. Dendritic cell–derived IL–2 production is regulated by IL–15 in humans and in mice. *Blood.* 2005 Jan 15;105(2):697–702.

Foti M, Granucci F, Ricciardi–Castagnoli P. A central role for tissue–resident dendritic cells in innate responses. *Trends Immunol.* 2004 Dec;25(12):650–4.

Francesca Granucci, Ivan Zanoni, Norman Pavelka, Serani L.H. van Dommelen, Christopher E. Andoniou, Filippo Belardelli, Mariapia A. Degli Esposti, and Paola Ricciardi–Castagnoli. A Contribution of Mouse Dendritic Cell–Derived IL–2 for NK Cell Activation. *J. Exp. Med.*, Aug 2004; 200: 287 – 295

# Innate Immunity and Disease Susceptibility

Prof. Ralf Schumann, University of Berlin, Germany

Innate Immune responses of the host are based on the direct recognition of foreign molecules leading to a rapid response including the release of pro-inflammatory cytokines. Recently, with the discovery of the family of toll-like receptors (TLRs) and their signaling pathways, key reaction patterns of Innate Immunity have been elucidated. TLRs are the signal transducing receptors for recognition of invading microorganisms, their cell wall compounds, such as Lipopolysaccharide (LPS) and for microbial nucleic acids. By “gain- and loss-of-function” experiments we have identified novel glycolipids isolated from spirochetes and parasite proteins as ligands for TLR-2. We have also shown that the ability of individuals to respond to TLR-ligands may be impaired by single nucleotide polymorphisms (SNPs) within TLR-genes resulting in an altered susceptibility or course of disease. We report that two co-segregating SNPs, Asp299Gly and Thr399Ile, within the gene encoding for TLR-4, the receptor for bacterial LPS correlate with susceptibility to several infectious diseases. On the other hand our results show that these SNPs may protect from atherosclerosis and related diseases. SNPs of genes encoding for other TLRs, i.e. TLR-2 recognizing a wide variety of microbial ligands have an impact on susceptibility to infectious and inflammatory diseases as well. Recently we have found that in an African population in an endemic region for malaria, frequent TLR-2 SNPs were completely absent while the TLR-4 SNPs were more frequent and correlated with malaria susceptibility confirming the importance of the innate immune system and their genetic variations for infectious disease susceptibility.

## **Molecular mechanisms that regulate the egress of immature B cells from the bone marrow and their splenic targeting**

Flaishon Liat, Hart G., Lantner F., Topilski I., Shachar, I.,  
Weizmann Institute of Science

B-cell development involves the ordered progression of a stem cell through a number of stages, ultimately resulting in a mature B cell. The first cells expressing IgM on their surface during this developmental process are the immature B cells, that leave the bone marrow for their final maturation in the spleen. These recirculating cells are sequestered from encountering foreign antigens present in lymph nodes or sites of inflammation, prior to their splenic arrival; however, the mechanism controlling this phenomenon has not been elucidated. Our studies show that immature B cells can actively exclude themselves from antigen-enriched sites. This homing regulation is mediated by two independent inhibitory pathways, both control cytoskeletal rearrangement required for promoting integrin-mediated adhesion and migration of B cells. The first pathway involves the secretion of IFN- $\gamma$ , which is transcribed and secreted at low levels by immature B cells. The second pathway is regulated by the chemokine receptor, CCR2, which is expressed on murine immature B cells and whose expression is downregulated in the mature stage. We further demonstrate that IFN- $\gamma$  regulates the egress of immature B cells from the BM. The BM niches that control B cell differentiation have been extensively studied; however, the pathways that regulate immature B cell release have not been characterized. We show that low dose IFN- $\gamma$ , secreted by immature B cells, perturbs the CXCL12-retention response in the BM, allowing the immature B cell egress from this compartment. Thus, autocrine regulation mediates immature cells release from the BM and their splenic targeting.

# Neurotrophic and Immunomodulating Tellurium Compound (AS101) Protects Dopaminergic Neurons in Parkinson's Disease Models

Benjamin Sredni<sup>1</sup>, Revital Geffen<sup>1</sup>, Yona Kalechman<sup>1</sup>, Wenzhen Duan<sup>2</sup>, Michael Albeck<sup>1</sup>, Frances Shalit<sup>1</sup>, Harry M. Lander<sup>4</sup>, Kinor Noa<sup>1</sup>, Sredni-Kenigsbuch Dvora<sup>1</sup>, Tali Sonino<sup>1</sup>, Dan L. Longo<sup>3</sup>, Mark P. Mattson<sup>2\*</sup> and Gal Yadid<sup>1</sup>

In Parkinson's disease (PD) dopaminergic neurons in the substantia nigra (SN) become dysfunctional and many ultimately die; oxidative stress, mitochondrial dysfunction and reduced support by neurotrophic factors are implicated in the disease process. We report that the tellurium immunomodulating compound ammonium tri-chloro(dioxoethylene-O,O')-tellurate (AS101) protects dopaminergic neurons and improves motor function in rat and mouse models of PD. AS101 acts directly on dopaminergic cells, stimulating Ras-mediated dopamine production and protection against neurotoxin-induced cell death. AS101 inhibits the production of interleukin-10 (IL-10) and IL-1b, while increasing the expression of glial cell line-derived neurotrophic factor (GDNF) and IL-6. The neuroprotective kinases Akt and mitogen-activated protein kinases are activated, levels of the anti-apoptotic protein Bcl-2 are increased, and the cell death proteases caspases 1 and 3 are inhibited by AS101. The neuroprotective and neurorestorative actions of AS101 in animal models of PD, together with its excellent clinical safety profile in humans, suggest that it has potential as a therapeutic agent for PD.

# Mechanisms of Signaling by the SLP-76 Adaptor Protein in the TCR Signaling Pathway

Yablonski Deborah, Gonen R., Beach D., Bogin Y., Rappaport Faculty of Medicine, Technion, Haifa

A heterotrimeric complex of adaptor proteins consisting of LAT, Gads, and SLP-76 is required to mediate the TCR-induced activation of PLC- $\beta$ 1. LAT, a transmembrane adaptor, is constitutively localized to a detergent-resistant fraction of the plasma membrane, known as glycosphingolipid-enriched membrane microdomains (GEMs). SLP-76 is a cytoplasmic adaptor protein, whose interaction with LAT is bridged by Gads. Mutational analysis has revealed that both SLP-76 and LAT make multiple contributions to the activation of PLC- $\beta$ 1, which will be discussed in my talk. Overall, our results support a model in which SLP-76 is required to mediate at least three steps in the activation of PLC- $\beta$ 1, including: (i) promoting the TCR induced translocation of PLC- $\beta$ 1 to the GEMs; (ii) mediating the activation of a tyrosine kinase capable of phosphorylating PLC- $\beta$ 1 and (iii) bridging the interaction between this tyrosine kinase and PLC- $\beta$ 1. Whereas the first two steps require SLP-76-mediated protein-protein interactions, the third step also requires a flexible linker sequence within SLP-76, which does not mediate any protein-protein interactions. The results paint a picture of adaptor proteins as dynamic signaling molecules that both nucleate signaling complexes and regulate the activity of the enzymes within the complex.

# **Oral Presentations**

**19 – 30**

## **Naturally occurring breakdown products of inflammation, generated in inflammatory sites, functioned as down-regulator of inflammation.**

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Inflammation usually occurs in extravascular tissues following the invasion of micro-organisms, such as bacteria, to the body. These sites also contain immuno-modulators (e.g., chemokines, cytokines, acute phase proteins), and leukocyte-derived extracellular matrix-specific enzymes, such as heparanase and elastase, which can modify the composition of the tissue, and probably also degrade certain inflammatory mediators, thus yielding putative bioactive products. We postulate that these new small molecular weight mediators can exert effector functions needed to evoke or terminate inflammation.

Herein, we identified novel immunoregulatory compounds in the degraded products of human body fluids, especially in wound fluids of chronic leg ulcers of diabetic patients. Thus far we were able to isolate and identify several amino acid sequences and synthesized, and examined them *in vitro* and *in vivo*. Among these peptides, two are derived from apolipoprotein A-1 and two from fibrinogen. Treatment of purified human T cells with these peptides down-regulated nuclear factor- $\kappa$ B activity and reduced the secretion of TNF- $\alpha$  and interferon- $\gamma$ . *In vivo*, these peptides markedly inhibited DTH reaction, ConA-induced hepatitis and inflammatory bowel disease (IBD) in mice. We suggested that these peptides might terminate inflammatory reactions by transmitting negative signals to the inflammation-inducing leukocytes. Our results indicate that by using this approach we may have found a first set of such novel anti-inflammatory peptides. We hope that such peptides can be used to down-regulate inflammatory reactions *in vivo* in human patients suffering from chronic inflammatory diseases.

## DNA microarrays analysis in search of new drug targets for myasthenia gravis

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Myasthenia gravis (MG) and its animal model, experimental autoimmune MG (EAMG) are autoimmune disorders in which the acetylcholine receptor (AChR) is the major autoantigen. DNA microarray technology, supported by quantitative real time PCR, immunohistochemistry and flow cytometry, were used to identify new potential drug targets for MG and to delineate genes involved in the pathogenesis of MG.

1. The chemokine IP-10 and its receptor CXCR3 were found to be over-expressed in LNC and muscles of EAMG rats and in thymuses and muscles of MG patients. CXCR3 was up-regulated in CD4+ T cells of MG patients.

2. The expression of several phosphodiesterase (PDE) subtypes was up-regulated in LNC and muscles of EAMG rats. Pentoxifylline (PTX), a general PDE inhibitor, suppressed the progression of EAMG when treatment started at the acute or chronic stages of disease. Suppression was associated with down-regulation of humoral and cellular AChR-specific responses and with down-regulation of specific PDE subtypes and up-regulation of Foxp3, a transcription factor essential for CD4+CD25+ regulatory T cell function.

3. Data mining of the muscle transcriptome revealed two major groups of deregulated genes common to MG and EAMG: a) Genes linked to muscle biology including muscle proteins regulating contraction such as myosin polypeptides and myosin binding proteins; 2) Genes coding for the chaperone protein category including several heat shock proteins. There were no inflammation-associated deregulated genes in MG or EAMG.

Our results demonstrate the power of DNA microarray technology to identify novel genes involved in the pathogenesis of myasthenia and other autoimmune diseases.

## Enhanced Tumor and Virus Spread in Ncr1 (NKp46) Deficient Mice

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The elimination of viruses and tumors by Natural Killer (NK) cells is achieved by specific activating receptors. However, the direct in vivo role of these activating receptors was never investigated. To study the direct function of the major activating NK receptor, NKp46 (Ncr1 in mice) we replaced the Ncr1 with the GFP reporter gene. Enhanced spread of certain tumors was observed in the Ncr1 deficient mice and influenza virus infection was lethal in the absence of NCR1. Accumulation of NK cells could be observed at site of infection by using the in vivo GFP labeling, a powerful analytical tool which marks NK cells only. Our results reveal a critical role for Ncr1 in the in vivo elimination of tumors and especially of influenza viruses.

## The IL-10 gene as a model for molecular regulation of cytokines

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Cytokines are potent regulatory mediators of the immune system. Stimulation of different combinations of cytokines plays a major role in determining the character of immune responses and can result in variable degrees of pro- and anti-inflammatory effects. Several transcription factors have been found to participate in cytokine activation, such as NF $\kappa$ B. In this study we set out to reveal novel elements which mediate cytokine upregulation. We chose IL-10 as a model gene, and our initial assay was to detect changes in protein binding to its promoter following stimulation of transcription. Using the electromobility-shift assay (EMSA) we found a discrete region, proximal to the transcription start site, that exhibited intense binding of protein extracted from lipopolisaccharide(LPS)-treated cells as compared to naïve cells. Mutations that disrupted this binding defined the recognition sites as three repeats of a 5bp element. We next examined the functional significance of these elements by transfection experiments. The IL-10 promoter was cloned upstream to the luciferase reporter gene, and transfected into Hut-78 cells, which constitutively express IL-10. This set of experiments demonstrated a reduction in promoter activity following introduction of mutations into the 5bp elements. Comparison of human and mouse promoters disclosed a nearly complete identity of the 5bp repeats, supporting their regulatory importance. Furthermore, these sequences were found to be abundant in the promoters of other cytokines. Preliminary results obtained from a gene array analysis indicate that promoters harboring the 5bp element may be more susceptible to induction of expression.

Finally, our work on the IL-10 promoter revealed novel regulatory elements, which may be of relevance in other cytokines as well. Further work is needed to characterize the trans-acting protein factor which recognizes these binding sites.

## Staurosporine Sensitizes Leukemia Cells to Glucocorticoid-Induced Apoptosis

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Glucocorticoids (GCs) are commonly used for therapy of lymphomas and leukemias, due to their ability to induce apoptosis. While certain leukemic cells are sensitive, others are resistant despite expressing the glucocorticoid receptor (GR). In the present study, we have searched for agents that affect GC-induced apoptosis. We observed that sub-toxic concentrations of staurosporine (STS), a broad-spectrum kinase inhibitor, convert GC-resistant B10 and S49 T lymphoma cells into GC-sensitive ones. STS sensitization to GC depends on functional GR expression since the GR antagonist RU-486 abolished the STS effect and GR-deficient lymphoma cells were refractory. We found that STS redirects GR translocation to the mitochondria in Dex-treated B10 cells and alters the reactivity of native GR to the N-terminal reacting M20 antibody. As GR translocation to the mitochondria is essential for GC-induced apoptosis, the STS effect on GR trafficking may contribute to GC sensitization. The Cdk2-inhibitor roscovitine offsets the effect of STS on B10 cells, suggesting a role for Cdk2 activation in this process. Furthermore, combined treatment with STS and Dex induces the expression of the orphan receptor Nur77, a pro-apoptotic protein. Interestingly, STS also induces a higher molecular weight variant of the pro-apoptotic protein Bim in B10 cells. Altogether, our data show that STS acts upon various pro-apoptotic proteins, which in turn may increase the apoptotic sensitivity of leukemia and lymphoma cells to GC. Identifying pro-apoptotic proteins that are targeted by STS is crucial for effective sensitization of leukemia cells to GC-induced apoptosis.

## Cell Surface Microdomains and the Assembly of Immunological Synapses

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The “immunological synapse” (IS) is the contact area of cell–cell conjugates, such as T cells and antigen presenting cells (APC), where information is transferred from cell to cell via the spatial segregation of clusters of proteins. The rate of accumulation of experimental information necessitates the use of integration tools, i.e., mathematical and computational modeling, in order to evaluate the potential mechanisms underlying IS formation and function. We created a stochastic cellular automaton simulation to study T cell–APC IS formation. Using this simulation, we offer the following insights. (i) The initial locations of molecules of each type at the moment of contact are responsible for the formation of the “immature” IS, displaying adhesion molecules in the center surrounded by a peripheral ring of TCRs. (ii) Movement of large clusters of TCRs (for example, in lipid rafts) towards the center of the contact area accelerates the inversion of the immature synapse into a mature synapse (i.e. with TCRs in the center and adhesion molecules in the periphery).

# **Monocyte Apoptosis Induces Expression and Secretion of a 26 kDa Protein that Mediates Engulfment of Apoptotic Monocytes and Immune Suppression of Immature Dendritic Cells**

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Apoptotic cells have been shown to signal their neighbors in a variety of ways. “Eat me” signals expressed on apoptotic cells serve as markers for phagocyte recognition and subsequent apoptotic cell ingestion. “Do not eat me” signals, present on viable cells, are down-regulated on apoptotic cells, and have the opposite effect. Apoptotic cells can also generate “find me” signals by release of membrane molecules such as lysophosphatidylcholine, which is important for phagocytic cell recruitment. In the current study we applied a proteomic approach to identify molecules that are secreted from apoptotic monocytes and as such are candidate for mediating engulfment and immune suppression. Supernatants of monocytes undergoing serum withdrawal apoptosis, in the presence and absence of the pan caspase inhibitor zVAD-fmk, were collected and compared using 2D SDS-PAGE separation. Differentially displayed proteins were then isolated, processed and identified using tandem mass-spectrometry technology. Using this methodology, one of the proteins identified was P12, a 26 kDa protein, that is expressed upon induction of apoptosis. Using a model of interaction with immature dendritic cells (iDCs), we validate that P12 mediates direct and indirect engulfment, as well as immune suppression. Consequently, we conclude that P12 represents the first identified protein, generated and secreted by apoptotic monocytes, that signals iDCs to improve engulfment and to tolerate engulfed material.

# The Involvement of Fractalkine in Bone–Marrow–Endothelium Transmigration of Neuroblastoma Cells

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Neuroblastoma (NB) is the most common solid tumor in children. The bone marrow is a favorable site of metastasis formation by NB cells which have to transmigrate through the bone marrow endothelium in order to metastasize into this site.

Transendothelial migration (TEM) of tumor cells is a crucial step in metastasis formation, postulated to be similar to that of leukocyte TEM. This process involves chemokines and adhesion molecules.

Most chemokines are secreted as soluble molecules and are presented on glycosaminoglycans to retain a local chemokine gradient. Fractalkine (CX3CL1), in contrast, is a unique membrane bound chemokine that functions also as an adhesion molecule and can be cleaved to a soluble fragment, capable of attracting fractalkine receptor (CX3CR1) expressing cells.

In the present study we asked if CX3CL1 and CX3CR1 are involved in neuroblastoma–TEM. We demonstrated for the first time that both CX3CL1 and CX3CR1 are expressed by several neuroblastoma cell lines.

The differential expression of CX3CL1 either as a transmembrane or as a shed chemokine in SH–SY5Y NB cells was found to be regulated by PKC activation.

Further experiments demonstrated that CX3CL1 and its receptor expressed by NB cells are active. For example, CX3CL1 attracts CX3CR1–expressing neuroblastoma cells to transmigrate through bone marrow endothelial cells, and both CX3CR1 and CX3CL1 can transduce signals into neuroblastoma cells.

Based on these results, our working hypothesis is that CX3CL1 and CX3CR1 play a functional role in neuroblastoma–endothelium interactions and metastasis formation.

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## **Native and fragmented fibronectin oppositely modulate monocyte secretion of MMP–9**

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Monocytes remodel the ECM by secreting both proteins composing it, e.g. fibronectin (FN), and proteases, e.g. matrix metalloproteinase–9 (MMP–9), which cleaves FN into fragments. FN may induce MMP–9 expression, but the effects of fragmented FN on monocyte MMP–9 are unknown. We show that TNF± induces both MMP–9 and fragmentation of FN into mixture of inseparable but distinct fragments. To mimic their encounter with the inflammatory ECM, primary monocytes or the U937 monocytic cell line were incubated on a plastic substrate, plastic coated with native FN, and plastic coated with MMP–9 fragmented–FN. Native FN inhibited secretion of TNF±–induced proMMP–9 by 2–fold (p

## A new intranasal influenza vaccine based on a novel polycationic lipid – CCS: from the bench to the clinic

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A mucosal vaccine, which can be self-administered without injection, may provide both effective systemic and mucosal immunity. It could also be used expediently for mass vaccination in case of a sudden epidemic or a bioterror attack.

We describe herein a novel polycationic sphingolipid (ceramide carbamoyl–spermine = CCS), having combined carrier and adjuvant activities. In combination with viral antigens CCS elicits, in mice and rabbits, strong systemic (serum) and local (in the nose and lungs) humoral responses, as well as cellular responses and protective immunity following intranasal (i.n.) administration. In an Influenza model in mice, the vaccine formulated with CCS in the form of unsized heterogenous liposomes was up to 10,000–fold more immunogenic than the standard vaccine. High antibody titers and protective immunity to viral challenge persisted for >9 months following vaccination. Furthermore, this vaccine was highly efficacious following a single dose (i.n., i.p., or i.m.), given without adjuvant, in both young (2 Mo) and old (18 Mo) mice, and it elicited high titers of strain cross-reactive hemagglutination inhibiting (HI) antibodies. Biodistribution studies revealed that the i.n. CCS vaccine (lipid and protein) was retained for >24h within the upper respiratory tract with no leakage to the brain. Adjuvanticity of the CCS formulation could also be related to its action as a moderate "danger signal". No systemic adverse effects, and only minimal local inflammation were observed in mice and rabbits. A phase I/IIa clinical trial with the i.n. CCS–flu vaccine is underway.

## **Direct and Immune–Mediated Antitumor Activity of Newcastle Disease virus (NDV)**

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Viruses may have both direct cytolytic and immunomodulatory effects, which may be valuable in cancer treatment. In the present study we explored the effect of NDV on the development of B16 melanoma in C57Bl mice.

Injection of NDV–infected B16 cells into mice, fully suppressed the ability of the B16 cells to develop tumors. NDV does indeed infect B16 cells in vitro, inhibit their proliferation by G1 arrest and ultimately induce apoptotic cell death, associated with decline in Bcl–xL expression. These results suggest direct killing effect of NDV on B16 cells.

The immune–mediated antitumor effect was clearly demonstrated as strong and significant retardation of tumor development in mice treated by NDV infected B16 cells before or after challenge with uninfected B16 cells. A heavy mononuclear infiltrate was observed within the tumor and around it, with prominent presence of macrophages. Moreover, tumor development was significantly inhibited when a mixture of B16 cells and spleen cells of mice treated by NDV infected B16 cells were transplanted to mice. In vitro, NDV induced increased expression of MHC–I, LFA–1 and ICAM–1 molecules on B16 cells and a parallel increase in key surface molecule (MHC I and II, CD40 and FcR) expression in a macrophage cell line (RAW 264.7). Furthermore, NDV caused elevation in the size and quantity of lysosomes in the RAW macrophages, as well as increase in TNF $\alpha$  and NO production. Supernatants of NDV infected RAW cells caused inhibition of B16 proliferation and G1 arrest. Our results indicate that NDV has direct antitumor effect, most probably by induction of apoptosis. In addition, NDV induces strong immune activity against the tumor, part of which may be related to increased expression of relevant recognition, adhesion and probably tumor associated surface molecules. Macrophages seem to be strongly involved in the NDV– induced antitumor effect.

## **Sustained T cell motility and productive DC encounters triggered by matrix bound rather than by soluble chemokines**

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T cell zones in peripheral lymph nodes contain high and uniform levels of the key T cell and dendritic cell (DC) chemokine CCL21. Recent in vivo imaging suggests that T cell motility in this zone is random, but functional linkage between CCL21 and this motility has been missing. We show that extracellular matrix-immobilized forms of CCL21 or CCL19 promote rapid T cell motility that persists for hours, whereas the soluble forms of these chemokines trigger poor and transient motility. Interstitial motility of T cells on extracellular matrices is thus robustly triggered by immobilized but not by soluble chemokines. Strikingly, lymphocyte motility is integrin-independent and both VLA-4 and LFA-1 are inactive. Nevertheless, T cells encountering mature DCs establish minute long contacts with these cells through their uropods and are independent of TCR activation. Inactive LFA-1, and its adaptor talin are highly enriched in these uropods, and these regions are potential nucleation sites of de novo T cell-DC immune synapses. This is the first evidence that signals from matrix presented chemokines can be integrated by locomoting lymphocytes to successfully encounter DCs recently arriving in the T cell zones and localize LFA-1 machineries within nascent immune synapses.

## **Poster Presentations**

**31 – 81**

## **Galectin 8 – a novel proapoptotic ligand of CD44**

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In this work we demonstrate, using affinity chromatography, surface plasmon resonance and flow cytometry, that galectin–8 is an additional ligand of CD44. With the aid of flow cytometry and the reverse–transcriptase polymerase chain reaction, as well as, nucleotide sequencing, we show that synovial fluid cells derived from rheumatoid arthritis (RA) patients contain galectin–8, cell surface CD44 and fibrinogen. All three molecules, possibly released from the inflamed synovium, were detected by Western blot and co–precipitation in the joint fluid of RA patients. Using the latter technology, as well as double staining flow cytometry, we further revealed that at least part of galectin–8, soluble CD44 and fibrinogen form a triple complex in the RA synovial fluid which reduces the ability of galectin–8 to induce apoptosis in RA inflammatory joint cells. Hence, the RA model not only confirms the receptor–ligand relationship between CD44 and galectin–8, but also unveils the biological significance of this interaction in regulating the inflammatory cascade.

## **The intracellular esterases activity of live cells may distinguish metastatic from tumor-free lymph nodes.**

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One of the major clinical problems in breast cancer is the relatively high incidence of occult lymph node metastases undetectable by standard procedures. Since the ascertainment of breast cancer stage determines the following treatment, such “hypo–diagnosis” (“sub–diagnosis”) leads to inadequate therapy, and hence, is detrimental for the outcome and survival of the patients.

The purpose of our study was to investigate functional metabolic characteristics of living cells derived from metastatic and tumor-free lymph nodes of breast cancer (BC) patients. Our methodology is based on the ability of the living cells to hydrolyze fluorescein diacetate (FDA) by intracellular esterases and on the findings the association of FDA hydrolysis rates with a specific cell status, both in physiological and pathological conditions.

Presented study demonstrated the significant difference in the ability to utilize FDA by lymph node cells derived from metastatic and tumor-free lymph nodes in general, and in the metastatic and tumor-free lymph nodes of individual patient as well. Cells from the metastatic lymph nodes had higher capacity to hydrolyze FDA, and increased this activity following additional activation by autologous tumor tissue (tt). The associations between FDA hydrolysis rate and some physiological cell parameters were shown.

The results of the present study may be helpful in predicting the risk of involvement of standard “tumor-free” axillary lymph nodes in occult metastatic process, and for reducing false–negative results of axillary examination.

## The two isoforms of CD58 associate with protein kinases in distinct membrane compartments

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The adhesion molecule CD58 is natively expressed in both a transmembrane form and a glycosylphosphatidylinositol (GPI)-anchored form, and hence provides a model for the study of two distinct membrane-anchored forms of the same protein in the same cell. We demonstrate here that the two isoforms of CD58 are localized in distinct membrane compartments. The GPI-anchored form localizes in lipid rafts, while the transmembrane form resides in non-raft domains. However, following cross-linking, a fraction of transmembrane CD58 redistributes to lipid rafts.

Cross-linking of CD58 triggers a substantial increase in kinase activity, which is associated with CD58 in raft microdomains and more predominantly with transmembrane CD58 in non-raft microdomains. A substantial fraction of the phosphorylation, associated with cross-linked CD58, is attributed to tyrosine kinases, since tyrosine kinase inhibitors mediate significant diminution in CD58 associated phosphorylation in raft and non-raft microdomains. Further analysis revealed that following ligation of CD58, Syk tyrosine kinase and Lyn tyrosine kinase become associated with CD58 in raft and non-raft microdomains.

The extensive inducible kinase activity, associated with transmembrane CD58, is demonstrated in wild-type cells that express the two isoforms of CD58, as well as in GPI-deficient variant cells which express only the transmembrane CD58.

Thus, the findings of this study suggest that CD58 may trigger signaling not only in raft microdomains, but also in non-raft microdomains, and that in spite of its exclusion from rafts, transmembrane CD58 transmits signals independently of the GPI-linked isoform.

## Involvement of the CD44 molecule in type I diabetes of NOD mice

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CD44 is widely expressed cell adhesion molecule that has been implicated in a variety of biological processes including leukocyte extravasations at inflammatory sites and tumor metastasis. Type I diabetes (IDDM) is a T cell–mediated autoimmune disease that implicates the insulin–producing  $\beta$  cells in the pancreatic islets. Because CD44 is associated with the migration of inflammatory cells into the pancreatic islets and their interaction with the islet matrix and endogenous cells, we decided to study its involvement in experimental IDDM in non–obese diabetic (NOD) mice by exploring the anti–diabetogenic effect of monoclonal antibodies (mAbs) directed against CD44 constant epitopes. We have shown that, in this animal model, CD44 and hyaluronic acid (CD44 principal ligand) targeting by specific antibody and enzyme, respectively, confers appreciable resistance to diabetes (PNAS 2000, 97: 285–290).

Since the effect of antibodies may be indirect, we have generated CD44–deficient NOD mice for exploring the biological role of CD44. Diabetes development was monitored in those mice, in both spontaneous and adoptive transfer models. We show here that CD44–deficient mice develop spontaneous diabetes significantly later ( $p=0.05$ ) than wild–type NOD mice do. Moreover we found in in vitro assays significant differences between wild–type and CD44–deficient NOD mice in proliferation of spleen cells after stimulation with insulin and in their migration pattern. Wild–type irradiated NOD males reconstituted with wild–type or CD44–deficient splenocytes by adoptive transfer develop diabetes while CD44–deficient irradiated NOD males reconstituted with wild–type or CD44–deficient splenocytes show significant resistance to diabetes development ( $p$

# The Effect of Genetic Polymorphism on Susceptibility to Type 1 Diabetes

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Type 1 Diabetes (T1D) is a multifactorial polygenetic autoimmune disease. It is caused by destruction of pancreatic  $\beta$  cells. The major susceptibility genes are found within the HLA class II region. A second genetic susceptibility locus has been mapped by a variable number of tandem repeat (VNTR) in the insulin gene (INS). In the present study we focused on the role of INS VNTR in combined with HLA in the etiology of T1D studying 4 Israeli ethnic groups: Yemenite, Ashkenazi, Ethiopian Jews and Israeli. Since the Yemenite Jews have an unusual gradual increase in the incidence of T1D, and the environmental factors are similar for all groups in Israel, it is clear that mainly genetic factors must be responsible for this difference. Our results show that in the Ashkenazi and Ethiopian Jews about 75%–85% of the T1D patients and 50% – 60% of the controls are homozygous to INS VNTR I alleles. However, the Yemenite T1D patients and healthy controls have significantly higher frequencies of INS VNTR I/I genotypes. In contrast to the Yemenite Jews in the Israeli Arab population there were no significant differences in the INS VNTR genotype frequencies between the T1D patients and the controls. We combined the INS VNTR results with the HLA genotyping in order to determine whether this combination improves risk assessment. Our results indicate that the HLA susceptibility and protective effects over comes the effects INS VNTR induces. This study demonstrates that the genetic factors that modify T1D risk are ethnic dependent.

## Studying Mononuclear Phagocyte In Vivo Functions

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The mononuclear phagocyte (MP) system is a body wide network of myeloid cells that is crucially involved in tissue homeostasis as well as the innate and adaptive immune defense. In order to begin to functionally dissect the macrophage (MF) and dendritic cell (DC) network in the intact organism, we recently developed a novel diphtheria toxin (DTx)– based experimental approach that allows the conditional in situ ablation of MP. The use of these mice allowed us previously to establish the crucial role of CD11c<sup>high</sup> DC in the priming of cytotoxic T cell responses against intra–cellular bacteria, viral and parasitic pathogens.

Here we will present our studies aiming to define of the APC capable of priming naïve T cells in the lymph nodes of immunized mice. Our results suggest that, surprisingly, Plasmacytoid DC (PDC) can compensate for the absence of “classical” CD11c<sup>high</sup> DC with respect to CD4, but not CD8 T cell stimulation.

In an independent set of experiments we are using a Cre/loxP–based binary transgenic system to direct DTx–receptor expression to the CD11c<sup>high</sup> CD8<sup>+</sup> DC subset. Due to their unique capacity to cross–present exogenous antigens in the context of MHC class I these cells are currently suspected to play a critical role in the maintenance of immunological tolerance. However, this notion awaits solid experimental evidence. Finally, we will report on our recent progress to achieve long term MP ablation and generate mice that constitutively lack DC.

## Antiviral effect of AS101 on West Nile Virus

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The immunomodulator AS101 [ammonium trichloro (dioxyethylene 0-0')tellurate] has an antiviral effect in vivo and in vitro. West Nile virus (WNV) is a mosquito-borne virus which has recently caused major human outbreaks in North Africa, parts of Europe, Israel and the USA. Infection of neonatal mice with West Nile virus (WNV) causes fatal damage to the central nervous system, and completely destroys Vero cells after 5–7 days by cytopathic effect (CPE). Our study aimed to determine whether AS101 has an antiviral effect against WNV. Vero cells infected with WNV (MOI=5\*10<sup>-4</sup>) and treated with AS101 (0.8ug/ml, 1.7ug/ml), showed 71%–69% protection of CPE. Moreover, supernatants collected from AS101 treated cells did not contain any viral RNA, as revealed Real-Time RT-PCR examination. In addition there was virus envelope protein (ENV) expression decrease, in WNV (MOI=5) infected Vero cells, after AS101 treatment (0.8ug/ml, 5ug/ml). Treating infected mice with AS101 (10mg/mouse) twice following WNV infection, conferred 60% protection on day 8, while no unprotected animals survived. Thus, the results presented here suggest that AS101 has an antiviral effect against WNV in vitro and in vivo.

## Protective effect of the tellurium compound AS101 in Fulminant Hepatic Failure

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Fulminant Hepatic Failure syndrome (FHF) induced by Lypopolysaccharide in Propionibacterium acnes primed mice involves inflammation leading to severe injury of hepatocytes, apoptosis and massive necrosis. The Tellurium compound AS101 has been recently shown to directly inhibit caspase-1 and 3 activity due to its ability to oxidize cysteine thiols within their active site. Therefore, due to its anti-inflammatory and anti-apoptotic properties, we aimed to investigate, in the present study, the effects of AS101 in this acute liver failure model.

In vivo administration of AS101 after the LPS challenge resulted in the inhibition of serum levels of ALT and AST. Histological evidence of the injury regarding massive necrosis and suggestive apoptosis also suppressed by AS101. Furthermore, AS101 attenuated the inflammatory process. This was expressed by decreased ICE activity, followed by lower levels of serum and intrahepatic IL-18 and IL-1b, – inflammatory proteins activated by ICE. This result may explain the lower levels of FASL in the AS101 treated group since IL-18 is a potent inducer of FASL in NK and lymphocytes and IL-1b was found to upregulate certain chemokines which trigger lymphocyte and macrophage invasion into the liver.

AS101 inhibited liver caspase-3 activity known to be a major mediator of both apoptotic and necrotic cell death. This event probably accounts for the decreased liver apoptotic lesions in mice treated by AS101 and the resulting improved functions.

Our results provide evidence that the protective effects of AS101 might be due to prevention of inflammation-induced apoptosis and lay credence to the potential importance of caspase inhibition in modifying the inflammatory and apoptotic response in this model. Moreover, therapeutic modulation of these processes by compound such as AS101 has the potential to alter the course of human liver disease.

## **Sensitization by AS101 of T and B lymphoma cells to chemotherapy via inhibition of the IL-10 – stat3 – survivin axis**

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The recently characterized novel member of the inhibitors of apoptosis (IAP) protein family, survivin, is potentially involved in both inhibition of apoptosis and control of cell division. Survivin is selectively expressed in most human neoplasmas and is involved in the chemo-resistant and radioresistant phenotypes of human tumors. On the basis of these findings, survivin has been proposed as a promising target for new anticancer interventions leading to human sensitization to chemical and physical agents.

IL-10 is constitutively secreted by a variety of human cancer cells, including lymphomas. This cytokine may control the activity of Stat3 and Akt – potent regulators of survivin. We showed that the immunomodulator AS101, a known anti IL-10 compound, can be used to target the survivin protein in T and B lymphoma cells. This occurs via disruption of the IL-10 autocrine/paracrine loop in these tumor cells resulting in the down regulation of the Stat3–Survivin axis. Our data showed that the inhibition of the IL-10–Stat3–Survivin signaling by AS101 is pivotal for sensitization of tumor cells to Taxol. This is reflected by enhanced caspase-3 activity and increased tumor cell apoptosis. pAkt, also inhibited by AS101, does not play a role in lymphoma cells sensitization by AS101. Furthermore, our data show similar sensitization of tumor cells to Abraxane, the new albumin-stabilized nanoparticle formulation of Taxol, designed to overcome insolubility problems encountered with Taxol, and facilitating the passage of the drug to the underlying tumor tissue.

Elucidating the mechanism of survivin repression by AS101 in cells of hematological tumors, and investigating the mechanism of tumor cell death ensuing from manipulation of the survivin pathway by AS101, may be relevant for novel therapeutic strategies to improve the efficacy of chemotherapy-induced apoptosis on patients.

## Nitric Oxide and Glucocorticoids produced by Thymic Epithelial Cells Synergize in Inducing Apoptosis of CD4+8+ Thymic Lymphoma Cells

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CD4+8+ thymic lymphoma cells (PD1.6) undergo apoptosis when co-cultured with thymic epithelial cells (TEC). We have previously shown that this apoptosis is mediated in part by glucocorticoids (GCs). However, recent evidence indicates that additional mechanisms are involved. Here we studied the role of NO $\cdot$  in this apoptotic process. We observed that 1,4-PBIT and L-NMMA, which inhibit the inducible isoform of nitric oxide (NO $\cdot$ ) synthase (iNOS), reduced the extent of TEC-induced apoptosis by 30%, suggesting an auxiliary role of NO $\cdot$ . RT-PCR revealed iNOS mRNA in TEC, but not in PD1.6 cells. The iNOS mRNA level was elevated in TEC after co-cultivation with PD1.6. Also, Western blot analysis demonstrated a corresponding increase in iNOS protein level in TEC. Using an NO $\cdot$  probe (DAF-FM diacetate), low amounts of the radical were detected in PD1.6 cells after co-cultivation with TEC. As both NO $\cdot$  and GC are produced at low concentrations by TEC, we asked whether they synergize in inducing apoptosis of PD1.6 cells. To address this question, PD1.6 were incubated with an NO $\cdot$  donor (sodium nitroprusside or S-nitrosoglutathione) alone or with Dex. We observed a synergistic effect on PD1.6 apoptosis. These data suggest that low amounts of NO $\cdot$  and GC produced by TEC synergize in inducing apoptosis of CD4+8+ cells. Since PD1.6 elevates iNOS expression in TEC, we hypothesize a paracrine mode of response, by which cytokines, produced by PD1.6 cells, elevate the levels of iNOS and NO $\cdot$  in TEC, which, in turn, enhances the apoptotic effect of GC on PD1.6.

## **CXCL8–induced migratory responses: The involvement of Paxillin and the regulation of its activation upon CXCL8 stimulation**

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CXCL8 is an inflammatory chemokine, inducing migration of hematopoietic cells to sites of acute inflammation. Of the different proteins involved in cell adhesion and migration, paxillin is a key regulator of focal adhesions and of cell motility.

The major aim of our study is to determine the involvement of paxillin in CXCL8–induced cell adhesion and migration, and to elucidate paxillin regulation upon cell activation by this chemokine. These issues were investigated in hematopoietic cells, transfected to stably express the CXCL8 receptor CXCR2.

Over–expression of a dominant negative mutant of paxillin gave rise to elevated levels of CXCL8–induced migration. This finding suggests that paxillin acts as a partial negative regulator of CXCL8–induced cell migration. Biochemical analysis has shown that cell exposure to migration–inducing concentrations of CXCL8 resulted in potent paxillin phosphorylation. This was regulated by carboxyl terminal domains of CXCR2, and was dependent on G $\beta$ i and G $\beta$ s. Potent CXCL8–induced paxillin phosphorylation also required activation of integrin–mediated signals, probably of  $\alpha$ 1 integrins. Moreover, our findings suggest that Focal Adhesion Kinase, but not Pyk2 and Src is involved in CXCL8–induced paxillin phosphorylation.

Overall, our findings suggest that CXCL8 regulates paxillin activity, possibly by controlling its phosphorylation, and that paxillin fine–tunes migration processes induced by CXCL8.

## Potential involvement of the CrkII adapter protein in T cell antigen receptor function

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T cell antigen receptor (TCR) cross-linking initiates the activation of biochemical cascades that transduce extracellular signals into the nucleus. Among the early biochemical events identified are the phosphorylation of tyrosine residues in the TCR  $\zeta$  chain immunoreceptor tyrosine-based activation motifs (ITAMs), and binding of ZAP-70 PTK to the phosphorylated  $\zeta$  chain. We found that significant amounts of tyrosine phosphorylated  $\zeta$  chains (pY- $\zeta$ ) coimmunoprecipitate with CrkII from a lysate of activated P116 T cells, which are devoid of ZAP-70. Coimmunoprecipitation of pY- $\zeta$  with CrkII was also observed in a heterologous system of Cos cells cotransfected with CrkII,  $\zeta$ , and constitutively active Lck (Y505F) expression vectors. Two-dimensional gel electrophoresis followed by immunoblotting confirmed that CrkII binds the tyrosine phosphorylated  $\zeta$  chain. In addition, using Far-Western blot analysis we found that binding is mediated via a direct physical interaction between the CrkII-SH2 domain and phosphotyrosyl-containing sequences on  $\zeta$ . High affinity interaction of ZAP-70 with activated  $\zeta$  is achieved through the cooperative interaction of its two tandem SH2 domains, with two adjacent phosphotyrosyl residues in each of the three individual ITAMs. Nevertheless, partial stimulation of T cells (leading to suppression, anergy, or tolerance induction) is known to result in partial phosphorylation of  $\zeta$ . We suggest that transient binding of CrkII to partially phosphorylated  $\zeta$  serves as a mean for protection of the  $\zeta$  chain from tyrosine phosphatases. In addition, by simultaneous interaction (via its SH3 domain) with proline-rich sequence-containing binding partners CrkII can recruit additional effector molecules to the partially phosphorylated TCR.

## **IL-1a translocate to the nucleus in different stress states and acts as a transcription factor.**

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Interleukin-1 alpha (IL-1a) is a potent pleiotropic cytokine that primarily affects inflammatory, immune responses, angiogenesis and hematopoiesis, but also other homeostatic functions of the body such as growth, differentiation and apoptosis. The potency of IL-1a stems mainly from its ability to induce expression of cytokines, chemokines, adhesion molecules and other pro-inflammatory molecules in a large diversity of cell types. Recent results in our lab have indicated that IL-1a translocate to the nucleus after activation with bacterial LPS, rTNFa or rIL1. We further described the activity of the N-terminus of IL-1a as a transcription factor using the GAL4 – luciferase method. Recent results demonstrated by immunofluorescence indicate that other stress signals, such as hypoxia and heat shock result in translocation of IL-1a to the nucleus. Protein interaction studies using the yeast two hybrid system have suggested several candidates for the target molecules of IL-1a in the nucleus. We are currently studying these proteins properties and the states in which they interact with IL-1a in situ. In order to further understand the role of intracellular IL-1a, we have checked the complete genome expression profile of IL-1a derived MEFs using affimetrix high density DNA chip. We have identified several clusters of genes that are differentially expressed in the absence of IL-1a both in homeostasis and after activation. Our findings suggest that proIL-1a, in addition to acting as a classical cytokine might be also active in the living cell as a stress related molecule.

## Identification and selection of disease related autoreactive T cells from early multiple sclerosis patients for specific T-cell vaccination

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CD4+ T cells sensitized against myelin immunodominant epitopes take part in the pathogenesis of MS. We are applying T cell vaccination, one experimental approach for treating MS, to probable MS (P-MS) patients following the first demyelinating attack. Patients are immunized with attenuated myelin responsive autoreactive T-cell lines.

Distinguishing disease-related autoreactive from normal myelin reactive lineages is essential for vaccine preparation as T-cell lines can also be generated from healthy subjects. Using microarray analyses we identified a unique autoimmune gene expression fingerprint in MS lines with overexpression of IGF-BP3, VEGF, BCL2 and Lifeguard. This imprint is absent from healthy subject myelin-responsive or MS patient tetanus toxin-reactive lines. BCL2 overexpression suggested resistance of MS lines to apoptosis. We used this property to generate MS T cell lines, 93-99% CD4+ oligoclonal (4-9 TcR Vbeta isoforms), IFNgamma producing cells bearing >90% CD45RO+ memory determinants. To characterize mechanisms differentiating MS from healthy cells we stimulated MOG responsive healthy subject T cell lines with the proinflammatory SDF1, TNFalpha or IFNgamma. Treated cells expressed higher IGF-BP3, VEGF and BCL-2 gene levels mimicking the autoimmune fingerprint. Autoimmune fingerprint-bearing lines were insensitive to additional cytokine stimulations implying a pre-existing hyperactivated state. While most P-MS lines express the autoimmune fingerprint and cannot be further stimulated with these cytokines, several P-MS MBP-reactive lines bear healthy T cell features (low expression of IGF-BP3, VEGF and BCL-2, inducibility with cytokines). Autoimmune expression fingerprint and line resistance to apoptosis enable distinguishing disease-related from healthy lineages within MS individuals and better line selection for TCV.

## **IRF-8 is obligatory for the expression of PML tumor suppressor gene in myeloid cells**

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IRF-8 transcription factor plays critical roles in interferon signaling during the innate and adaptive immune responses against pathogen infections and tumor malignancies. Interestingly, IRF-8 Knock-Out (KO) mice develop a syndrome similar to human Chronic Myelocytic Leukemia (CML). In humans, down regulation of IRF-8 expression was reported in CML and AML patients, and significant increase was correlated with remission following interferon- $\pm$  medication. These observations suggest a tumor suppressing role for IRF-8. Using DNA microarray analysis we have identified the Promyelocytic Leukemia (PML) gene as a transcriptional target of IRF-8. Furthermore, its mRNA and protein levels were significantly elevated following IFN- $\alpha$  and LPS stimuli of peritoneal macrophages in wild type but not in IRF-8 KO mice. The mechanism of this upregulation was further studied by analyzing the promoter region of PML using Luciferase reporter assays, EMSA, and CHIP. PML acts as a tumor suppressor that serves as a scaffold protein in nuclear bodies (NBs), whose disruptions are associated to leukemogenesis. Interestingly, macrophages extracted from IRF-8 $^{-/-}$  exhibited disrupted NBs that were reformed following IRF-8 transduction. Since IRF-8 is associated with CML and regulates PML we searched for correlation between the PML levels and the progression of CML in patients using Quantitative RT-PCR analysis of mRNA extracted from peripheral blood. The levels of both IRF-8 and PML were significantly lower in CML patients than in healthy donors. However, significant increase in the expression level of both IRF-8 and PML was noted in cDNA samples from CML patients that responded to a combined treatment with IFN- $\alpha$  and Glivec. Based on these novel observations, our working hypothesis is that the progression of CML is due to dysregulated expression of IRF-8 that affects the expression of downstream genes among which is the tumor suppressor gene, PML.

## Immune mechanisms of atherosclerotic risk in Sleep Apnea Patients

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Obstructive sleep apnea (OSA) constitutes an independent risk factor for cardiovascular morbidity. T cells are essential effectors in immune mechanisms perpetuating atherogenesis. We proposed that intermitted hypoxia and sympathetic activation, which are prominent features of OSA, could induce T cell activation that may be implicated in atherogenesis in OSA. Using flow cytometry and chromium release assays we found that T cells undergo phenotypic and functional changes in OSA patients. Thus compared to controls adherence to and cytotoxicity against Human Umbilical Vein Endothelial Cells (HUVECs) were 3-fold higher in magnet separated OSA gamma/delta T cells and could be abolished by anti-TNF-alpha-antibody. Preincubation of non-stimulated HUVECs with OSA gamma/delta T cells increased the adhesion of CD4+ and CD8+ lymphocytes to HUVECs by 2.7-fold. Increased cytotoxicity of OSA CD8 T cells was induced by CD56+/CD16+/Perforin+ subset and positively correlated with severity of hypoxemia and CD40L expression. Cytotoxicity of purified CD4 T cells was positively correlated with CD4+/CD28null population. Increased TNF-alpha expression in gamma/delta and CD8 T cells correlated with severity of OSA. Moreover a 2-fold increase in the % of TNF-alpha positive T cells was observed in OSA patients with cardiovascular disorders compared to only OSA. Nasal Continuous Positive Air Pressure (nCPAP) treatment that normalizes breathing in sleep significantly lowered the cytotoxicity, CD40L and TNF-alpha expression in T cells. We concluded that pro-atherogenic lymphocyte activation and increased cytotoxicity against endothelial cells may be involved in atherogenesis in OSA. nCPAP treatment ameliorate some lymphocyte dysfunctions and thus block some of the atherosclerosis pathways.

## Differences in malignancy between 3-MCA induced tumor cells derived from BALB/c and IL-1a KO mice

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The IL-1 family consists of two stimulatory proteins, IL-1a and IL-1b, and one antagonistic protein, the IL-1 receptor antagonist (IL-1Ra). We have used an experimental system of chemical induced carcinogenesis (3-MCA), in control and IL-1a<sup>-/-</sup> mice. IL-1a<sup>-/-</sup> mice developed tumors in the same kinetics or even faster than control mice. Tumor cell lines from 3-MCA-treated mice were established. These cell lines were injected into control recipient mice. Fibrosarcoma cell lines obtained from BALB/c mice exhibited rapid tumor growth, whereas cell lines from IL-1a<sup>-/-</sup> mice did not develop into tumors when injected into BALB/c mice. Fibrosarcoma cell lines were assessed for their angiogenic and invasiveness properties. To assess whether the immune system is involved in prevention of growth of cell lines from IL-1a<sup>-/-</sup> mice, tumor cells were injected i.f.p. into sub-lethally irradiated BALB/c mice, which resulted in tumor development in all mice. In non-irradiated mice, a significant influx of immune cells (T cells and macrophages) was observed at the site of IL-1a<sup>-/-</sup> tumor cells injection. Injection of cell lines derived from BALB/c lines, resulted in a less dense infiltrate. In addition, in IL-1a<sup>-/-</sup> mice, reduced activity of immune surveillance cells, such as NK, NKT, CTLs and LAKs, was observed. Collectively, these results suggest that in IL-1a<sup>-/-</sup> mice there is an impairment in the process of immune editing, enabling the outgrowth of immunogenic cell variants in overt tumors. Experiments are in process to characterize the role of host-derived IL-1a in inducing anti tumor immunity.

## The A3 adenosine receptor – a new target and biological marker in rheumatoid arthritis: data from Phase IIa Study

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Synthetic agonists to the A3 adenosine receptor (A3AR) induce anti–inflammatory effect in collagen and adjuvant induced arthritis. The molecular mechanism involves de–regulation of the Wnt and the NF–kB signal transduction pathways resulting in the inhibition of TNF–a and the induction of apoptosis of inflammatory cells. CF101, an A3AR agonist has been shown in Phase I studies to be well tolerated at therapeutic doses.

This study summarizes the interim analysis data of a Phase IIa study in RA patients. Study objectives were look at: a. the safety and preliminary efficacy of CF101 in patients with active RA and b. the correlation between response to the drug and A3AR expression in peripheral blood mononuclear cells (PBMNC) at base line. The trial is a multi–center (11 sites), randomized, double–blind, parallel group, in which CF101 was given orally twice daily at doses of 0.1mg, 1.0mg and 4.0 mg, for 12 weeks. The primary efficacy endpoint is the ACR20/50 response at week 12.

An interim analysis was performed after 66 out of a scheduled 84 patients completed 12 weeks of therapy. CF101 reduced disease activity, showing maximal response at 1 mg. At 12 weeks, 58%, 30% and 8% of the patients receiving 1 mg of CF101 achieved ACR 20, 50 and 70 responses, respectively. The respective mean percentage reduction in the number of tender and swollen joints at 12 weeks was about 80% in all dose groups. CF101 was well tolerated with no dose–limiting toxic effects. Analysis of A3AR expression at base line showed that response to CF101 was directly correlated to high receptor expression at baseline.

To conclude, CF101 showed a clinical response without dose limiting side effects in patients with active rheumatoid arthritis. A3AR levels may be a predictive surrogate marker of response to this therapy.

## Allelic exclusion of somatic hypermutation at the Ig $\lambda$ locus in vivo

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Developmentally programmed monoallelic demethylation and rearrangement represent important steps in the molecular process leading to allelic exclusion at the Ig $\lambda$  locus in B cells. By introducing pre-rearranged  $\lambda$  genes into their physiological position, the critical rearrangement step is bypassed and, as a result, it is possible to generate mice that produce B cells simultaneously expressing two different  $\lambda$  light chains. Double expressing B cells, however, still undergo monoallelic demethylation at the  $\lambda$  locus, and this allele is then the preferred substrate for somatic hypermutation in each cell, even though methylation itself does not directly inhibit the AID reaction in vitro. It thus appears that the epigenetic mechanisms that initially bring about monoallelic V(D)J rearrangement continue to play a role in the control of antibody diversity at later stages of B cell development.

## **Thiols advance the effect of the immunomodulator AS101 as a growth inhibitor of malignant T cells, by increasing AS101 uptake.**

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The immunomodulator ammonium trichloro(dioxoethylene-o,o')tellurate (AS101), an organotellurium nontoxic compound, has an antitumoral effect which was demonstrated in several preclinical and clinical studies. Our study aimed to understand the synergism between thiols and AS101 in its antitumoral activity on malignant T cells. AS101 decreased cell proliferation of the cutaneous T-cell lymphoma (CTCL) cell line MyLa. This activity was associated with a G2 arrest in the cell cycle with a similar arrest in Jurkat T cells. With the addition of the thiols, 2-mercaptoethanol or Cysteamine, an increase in the G2 arrest was observed even at lower concentrations of AS101. At higher concentrations of AS101, a shift of the cells into apoptosis was seen. Apoptosis was accompanied by a decrease of the mitochondrial membrane potential and an increase of the percentage of cells that express the initiator caspase (caspase-9) and the effector caspase (caspase-3). Other forms of thiols, including, glutathione (GSH), Cysteine or N-acetylcysteine (NAC), did not potentiate the effect of AS101. This is due to a difference in the charge of the compounds created between AS101 and the different forms of thiols. We quantified the AS101 within the Jurkat cells by evaluating the intracellular concentration of tellurium using scanning electron microscopy (SEM) and energy-dispersive spectroscopy (EDS) analysis. The addition of Cysteamine to AS101 significantly increased the concentration of tellurium within the cells. When the cells were grown in the presence of phenylalanine or leucine, two amino acids which are incorporated into the cell via the L-System, neutral  $\pm$ -amino acids transporter, a reduction in the apoptotic effect of AS101 in the presence of Cysteamine was seen. A similar decline in apoptosis was seen when using the L-system inhibitor, BCH. The results indicate that some thiols increase the antitumoral effect of AS101 by increasing its uptake into the cells, possibly through the L system.

## **Somatic mutations and activation–induced cytidine deaminase (AID) expression in established rheumatoid factor–producing lymphoblastoid cell line**

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**Aims:** The question whether Epstein–Bar virus (EBV) transformed lymphoblastoid cell lines (LCLs) exhibit somatic mutations in their Ig variable region genes (IgV) during in vitro growth was studied.

**Methods:** The sequences of the rearranged VH of an adult–LCL which secretes a monoclonal IgM rheumatoid factor (RF–line) and of the Vk genes of cord blood LCLs were determined.

**Results:** EBV infection of adult and cord blood lymphocytes induces a rapid induction of AID, a mutator responsible for somatic hypermutation (SHM) in the IgV. SHM were not found in the rearranged Vk of cord blood LCLs. By contrast, the rearranged VH gene of the RF–line, exhibited a low level of somatic mutations in culture. The mutations were preferentially targeted to the WRCH/DGYW hot spot motifs and biased for GC nucleotides, indicating that they were due to AID mediated SHM. One point mutation in the CDR1 of the VH of “non–antigen binding” RF clones, correlated with loss of antigen binding activity.

**Conclusions:** Induction AID expression and SHM in the rearranged VH of adult–LCL, may explain the occasional loss of antigen binding activity occurring in freshly established antibody secreting LCLs. In addition, our results support the possibility that AID may act as an oncogene, since the tumorigenic outcome of EBV infection in B cells, may be partly mediated by the induction of the mutatory activity of AID.

## **The CrkII adaptor protein interacts with ARAP1 (Arf GAP and Rho GAP with ankyrin repeat and PH domain)**

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The CrkII adaptor protein interacts with ARAP1 (Arf GAP and Rho GAP with ankyrin repeat and PH domain). Alice Givoni, Sigal Gelkop and Noah Isakov, Department of Microbiology and Immunology and the Cancer Research Center, Ben Gurion University of the Negev, Beer Sheva, Israel.

Crk adaptor proteins are involved in multiple signaling pathways and because of their lack of catalytic activity, many studies on Crk were aimed at the identification of their binding partners and determination of the physiological importance of these interactions. To identify additional Crk binding proteins in human T cells we used bead-immobilized GST-CrkII and pulled down proteins from a lysate of resting and activated Jurkat T cells. Protein mixtures were separated by gel electrophoresis, stained with colloidal Coomassie blue dye, excised, and digested with trypsin. The resulting peptides were analyzed by LC-tandem mass spectrometry (MS/MS) and assigned to specific proteins with the Mascot search engine. A prominent Crk-binding partner was identified by LC-MS/MS as the product of the Arf GAP and Rho GAP with ankyrin repeat and PH domain (ARAP1). This protein serves as a potential effector molecule in multiple signaling pathways and is a regulator of several GTP-binding proteins that are involved in cell adhesion processes. Pull-down assays and coimmunoprecipitation studies confirmed the association between CrkII and ARAP1 in lysates of Jurkat T cells. Preliminary studies suggest that the SH3N and SH2 domains of CrkII are involved in the interaction with ARAP1, which occurs in resting cells and is further augmented following cell activation.

# Novel Functions of Human NK cells Revealed by High-Throughput Proteomic and Genomic Analysis

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Human NK cells play a critical role in the systemic host defense against pathogens and tumor cells. However, the abundance of different NK subsets in secondary lymphoid organs and maternal decidua, suggests that these cells might exert additional yet-uncharacterized intriguing functions. By applying high-throughput genomic and mass-spectrometry based proteomic analysis on NK subpopulations; we identified a number of protein groups that were not previously described in these cells. In depth functional characterization for some of these molecules established two novel functions for human NK cells.

First, proteomic analysis of unactivated and activated human NK cell membrane-enriched fractions demonstrated that activated NK cells can efficiently stimulate T cells, since they upregulate MHC class II molecules and multiple ligands for TCR costimulatory molecules in vitro and in vivo upon stimulation. Furthermore, by manipulating antigen administration, we show that NK cells possess multiple independent unique specific pathways for antigen uptake. These observations suggest novel APC-like activating functions for NK cells.

Second, we demonstrate that decidual NK cells obtained from maternal uterine mucosa during pregnancy regulate fetal trophoblast invasion and placental vascular remodeling in vitro and in vivo by production a unique array of chemokines and pro-angiogenic factors. Remarkably, such functions are regulated by interactions between decidual NK cell specific activating and inhibitory receptors and their ligands, uniquely expressed at the fetal-maternal interface. This large scale analysis followed by functional verification suggests a new unexpected link between the immune system and human reproductive tissue remodeling.

## **Tight regulation of IFN- $\gamma$ transcription and secretion in immature and mature B cells by the inhibitory MHC class I receptor, Ly49G2**

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In order to complete their maturation and to participate in the humoral immune response, immature B cells that leave the bone marrow are targeted to specific areas in the spleen, where they differentiate into mature cells. Previously, we showed that immature B cells actively down regulate their integrin-mediated migration to lymph nodes or sites of inflammation, enabling their targeting to the spleen to allow their final maturation. This inhibition is mediated by IFN- $\gamma$ , which is transcribed and secreted at low levels by these immature B cells, and is downregulated at the mature stage. The activating MHC class I receptor, Ly49D, which is expressed at high levels on immature B cells, stimulates this IFN- $\gamma$  secretion. Here we show that B cells co-express the inhibitory MHC class I receptor, Ly49G2. In addition, we demonstrate a tight regulation in the expression of the Ly-49 family members on B cells that depends on their cell surface levels. High levels of Ly49G2 have a dominant inhibitory effect on Ly49D expressed at low levels on immature bone marrow and mature B cells, resulting in inhibition of IFN- $\gamma$  secretion. However, low levels of the inhibitory receptor, Ly49G2, co-expressed with high levels of the activating receptor, Ly49D on the immigrating immature B cells, enable the secretion of specific low levels of IFN- $\gamma$ . This expression pattern insures the inhibitory control of peripheral immature B cell to prevent pre-mature encounter with an antigen, while enabling entry to the lymph nodes during the mature stage.

## **Anti-tumoral activity of the immunomodulator AS101 in Multiple Myeloma**

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Multiple Myeloma (MM) is a clonal B-cell malignancy affecting both the immune and the skeletal systems. MM accounts for 10% of all hematological cancers. The immunomodulating compound, AS101, has been shown to have direct anti-tumoral properties in several tumor models. The present study examined the anti-tumoral activity of AS101 in MM cell lines and in a mouse model. Treatment of MM cells with increasing concentrations of AS101 induced a significant inhibition of cell proliferation which was also reflected in G2/M growth arrest, an effect associated with increased activity of the double-stranded RNA activated PKR. Longer incubation of MM cells with AS101 resulted in an increase in the early apoptotic cell population. Suppression of cell growth and apoptosis induction resulted in downregulation of pAkt, pStat3 and Survivin protein expression levels. We next examined the signal transduction involved in AS101 activation and found that AS101 reduces IL-6 levels in MM cells, which is considered the main growth and survival factor in MM. More important, mice transplanted with 5T33MM cells showed prolonged survival following AS101 treatment as compared to untreated mice. Our results indicate that AS101 may be candidate in clinical applications as an alternative or additional approach in the treatment of MM patients.

## **Methotrexate induces IL–10 production and inhibits nitric oxide secretion in mononuclear cells of rheumatoid arthritis patients**

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The mechanism by which low dose methotrexate (the gold standard treatment for rheumatoid arthritis) or combined therapy exert their anti–inflammatory effect in rheumatoid arthritis (RA) patients is still debated. It has been realized that an early appropriate treatment is critical for a good outcome for the patients. Unfortunately, there are patients who fail to respond to methotrexate (MTX) or its combinations with other drugs – which failure becomes apparent only after several months of treatment. Therefore the aim of the present study is to determine specific early events underlining the immunosuppressive mechanism of MTX (or combined) therapy in order to better predict their efficiency. Lately, the MTX immunosuppressive effect has been related to apoptosis, especially in active RA patients. Our study showed that MTX activity can be related to its induction of apoptosis with ROS involvement.

The present study uses different cytometric assays (including cytokine, ROS, NO, and apoptotic assays), at an individual cell level and at a cell population level. In the present research we show that MTX induces IL–10 secretion in Peripheral Mononuclear Cells derived from active RA patients (N=20). IL–10 is an anti–inflammatory immunoregulatory cytokine that shifts the immune balance toward Th2 profile, which is of great relevance to RA patients that are characterized by Th1 predominance. Additionally, we found that MTX inhibits the production of nitric oxide (NO) secretion in these patients. A high correlation is shown between MTX IL–10 induction and NO inhibition in active RA patients. Our data suggest that apoptosis induction by MTX may be primarily due to IL–10 production via modulation of oxidative stress. Our results may contribute to a better prediction of MTX efficiency for specific RA patients, based on assessing the apoptotic effect of MTX on the cellular immunological status.

# Natural Cytotoxicity Receptors of Natural Killer Cells: Analysis of Their Interaction with Heparin/Heparan Sulfate

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Lysis of tumor cells by NK cells is mediated via natural cytotoxicity receptors (NCRs) expressed by the NK cells. Three NCRs, NKp30, NKp44, and NKp46 molecules, were identified. We demonstrated that tumor-membrane heparan sulfate proteoglycans (HSPG) are involved in the recognition of tumor cells by NKp30, NKp44 and NKp46. Reduced recognition by all NCRs is observed toward CHO cells lacking membranal heparan sulfate and glypican-1-suppressed pancreatic cancer cells. All of the NCRs showed direct binding to heparin/heparan sulfate with different affinities. Basic amino acids predicted to be implicated in the heparin/heparan sulfate binding site on NKp46 were mutated. The mutated NKp46D2 proteins retained their virus binding capacity but reduced their binding to tumor cells with a 10 to 100 fold lower KD when tested for direct binding to heparin. Further more, we discovered a natural NKp30 isoform that lack the binding to heparan sulfate. Finally, we characterized and compared the epitopes on heparan sulfate recognized by NKp30 and NKp44. For all NCRs an 8/10mer heparin was able to inhibit their binding to HeLa cells and heparin. The N-sulfation on heparin was important for both NCRs binding while the 6-O sulfation was more important in the case of NKp44. Another difference between the 2 NCRs epitopes on heparin/heparan sulfate was observed using different antibodies to heparan sulfate in a binding inhibition experiments. Our results spot new evidences for the identity of the tumoral ligands for NCRs.

## **Oncogenic Ha–Ras transformation potentiates apoptosis, that correlates with suppression of a survival protein Akt and survivin by the Immunomodulator AS101**

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Mutations in genes encoding members of Ras family of small G proteins (p21) are present in 30% of all human tumors, making the Ras family the most widely mutated group of human proto–oncogenes. Previously, we found an organic Tellurium compound, AS101, that exhibits immunomodulatory activity and strong anti–tumor effects. Whether these properties can be exploited for cancer therapy was examined using NIH3T3 fibroblast v–Ha–Ras transformed and V–mos transformed NIH3T3 fibroblast cells. In this study AS101 appeared to selectively block ~90 % of the proliferative growth of v–Ha–Ras transformed cells, while not affecting the growth of V–mos transformed cells and non–transformed NIH3T3 fibroblasts. Furthermore, the growth inhibiting effect of AS101 was prevented when the activity of p21Ras was blocked by expression of a dominant negative mutant N116Y of the v–Ha–Ras or by treating Ha–Ras transformed cells with a farnesyl transferase inhibitor. The anti–proliferative effect of AS101 was accompanied by morphological changes typical of apoptosis i.e.: membrane blebbing, condensation of nuclear chromatin, and formation of apoptotic bodies. Investigating the role of ras–induced pathway in AS101–induced apoptosis we have found that AS101–induced apoptosis of v–Ha–Ras transformed cells correlated with suppression of Akt and survivin protein expression, activation of caspases, and induction of the cyclin–dependent kinase inhibitors p21Cip1 and p27Kip1. Interestingly, the extracellular signal–regulated kinase (ERK) pathway plays a G0/G1 arrest role in non–transformed cells; however, it plays a pro–apoptotic role in Ras–transformed cells in response to AS101 treatment. Thus, our results suggest that AS101 can be used to effectively treat tumors that bear an oncogenic mutation in p21Ras gene. [ and can selectively block the oncogenic function of mutated Ki/ Ha–Ras in human tumors]

## **Protective innate and specific immune response profile to streptococcus pneumoniae in mouse model**

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**Background:** The profile of CD4 T cells protective immune response to *S. pneumoniae* is unclear. An immunogenic pneumococcal surface protein with vaccine potential, Fructose Bisphosphate Aldolase (FBA), was used to study the nature of the protective immune response to *S. pneumoniae* in a mouse model system.

**Methods:** CD4+ T cells were obtained from C57BL/6 or BALB/c mice lymph nodes after immunization with rFBA prior and following intranasal challenge with *S. pneumoniae* serotype 3 strain WU2. The CD4+ T cells and rFBA treated antigen presenting cells, from naïve mice, were co–cultured. Proliferation level of CD4+ T cells and IL–2, IL–4, IL–5 and IFN $\gamma$  cytokine expression were measured by ELISA and Flow Cytometry.

**Results:** 2–doses of rFBA immunization elicited a significantly more effective CD4+ T cells proliferation than 1–dose. Pneumococcal challenge did not further enhance the proliferation of the CD4+ T cells. Moderate levels of IL–2 and IL–4 and high level of IFN $\gamma$  were detected by ELISA. IL–2, IL–4, IL–5 and IFN $\gamma$  expressing CD4+ T cells increased after 14 and 32 hours of incubation, as detected by Flow Cytometry.

**Conclusions:** Our results suggest that the protective immune response against *S. pneumoniae* elicited by rFBA was mainly of the TH2 type but with contribution of IFN $\gamma$  and IL–2.

# The Origin of Lung Mononuclear Phagocytes

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Blood monocytes are circulating precursors of mononuclear phagocytes that form a body wide network of myeloid cells involved in tissue remodeling, homeostasis and immune defense. Based on morphology, anatomic location and function mononuclear phagocytes have been subdivided into macrophages (M?) and dendritic cells (DC). Accordingly, lymphoid and non-lymphoid organs display characteristic M?/ DC compositions. Monocytes can differentiate in vitro into both M? and DC. However, in particular in the light of existing discreet monocyte subsets, it remains unknown what governs the in vivo monocyte fate.

The lung and alveolar space mononuclear phagocyte system is comprised of well-defined M? and DC populations, both in terms of function and surface markers. While CD11c+CD11b+ lung and alveolar DC were shown to initiate airway inflammations such as asthma, CD11c+CD11b- lung and alveolar M? seem to have an inhibitory influence. Here we report on the role of monocyte subsets as origin of lung and alveolar mononuclear phagocytes. Adoptive transfer studies of fractionated monocyte subsets allowed us to establish that both Gr1+ and Gr1- populations can differentiate into functional lung DC, albeit under distinct conditions, i.e. challenge and steady-state, respectively. We also show that Alveolar M? do not originate directly from blood monocytes, but from a resident proliferating precursor, presumably lung M?. In our experimental system these lung M? could only be derived from Gr1- monocytes, while the Gr1+ monocyte subset was destined to turn into DC. Our results suggest that the DC/ M? fate of the monocyte subsets is pre-determined, but controlled by environmental signals.

## **The Role of Antigen Processing and TCR: MHC Class II/peptide Interactions in the Manifestation and Th Polarization of Autoimmune Gastritis (AIG)**

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We examined the contribution of TCR: MHC/peptide (MHC/p) interactions and in vivo epitope availability to autoimmunity. Two TCR transgenic (Tg) mouse lines, A23 and A51, with specificity for two different I-Ad-restricted peptides from the  $\alpha$ -chain of the gastric parietal cell H-K/ATPase, were compared for the development of Autoimmune Gastritis. All A23 animals develop a Th1-mediated aggressive, inflammatory AIG early in life, while A51 Tg mice develop indolent Th2 mediated AIG at 6–8 weeks with only 50% penetrance. These phenotypes suggested that the A23 TCR had a higher avidity for MHC/p. However, A51 Tg CD4+ cells have significantly higher sensitivity to low doses of MHC/p in T cell proliferation assays. The two peptides displayed similar kinetic off-rates in binding assays with recombinant IAd. Staining of CD4+ T cells with IAd/p tetramers was only detected with cells from A51, consistent with a difference in TCR avidity. These results indicate that the A51 antigenic peptide binds efficiently to the MHC and its TCR displays a higher avidity for its cognate I-Ad/p. However, adoptively transferred, CFSE-labeled A51 T cells proliferated poorly in the gastric lymph node as compared to A23 T cells. Thus, the autoimmune potential of particular TCR is influenced not only by intrinsic affinity for their MHC/p complex, but also by the availability of the peptide antigen in the target organ.

## **Translocation of Active Heparanase to Cell Surface Regulates Degradation of Extracellular Matrix Heparan Sulfate, Upon Transmigration of Mature Monocyte–Derived Dendritic Cells**

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After antigen capture and exposure to danger stimuli iDC undergo maturation (mDCs) and migrate to regional lymph nodes where the presentation of antigenic peptides to T lymphocytes takes place. However, to migrate from peripheral tissue like the epidermis to regional lymph nodes, antigen–bearing epidermal Langerhans cells must move through extracellular matrix (ECM) of various compositions. The nature of their capacity to transmigrate via ECM is not well understood although MIP3b and CCR7 play a critical role. We were interested to verify whether heparanase, a heparan sulfate degrading endo– –D–glucuronidase, that participate in ECM degradation and remodeling, is expressed and functional in monocyte–derived DCs.

Using immunohistochemistry, confocal microscopy, RT–PCR, Western blot analysis, heparanase activity and matrigel invasion assays, we show that heparanase is expressed both in the nucleus and cytoplasm of iDCs, and that gene expression and synthesis take place mainly in monocytes and early iDCs. We further show that both nuclear and cytoplasm fractions show heparanase activity but upon LPS–induced maturation, heparanase translocates to cell surface and degrades ECM heparan sulfate. Matrigel transmigration assay showed a MIP3b comparable role of heparanase.

Heparan sulfate glycosaminoglycans play a key role in the self–assembly, insolubility and barrier properties of the ECM and the results of this study suggest that heparanase is a key enzyme in ECM transmigration of DCs.

# Adenosine is Induced During Peritonitis and Down Regulates Cytokine Production and Leukocytes Recruitment

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Adenosine is an endogenous immunomodulator that has been shown to exhibit anti-inflammatory and immunosuppressive effects. These anti-inflammatory effects depend mainly on the ligation with its cell-surface receptors subtypes: A1R, A2AR, A2BR, and A3R which are all G-protein coupled. The generation of extracellular adenosine involves phosphohydrolysis of adenine nucleotide intermediates and is regulated by two enzymes, nucleotidase triphosphate dephosphorylase (CD39) which converts ATP to AMP and 5' ectonucleotidase (CD73) which converts AMP to adenosine.

The aims of the present study were to elucidate the regulatory role of adenosine during peritonitis and to assess the regulation of CD39 and CD73 on peritoneal mesothelial cells (PMC) during the inflammatory processes.

In a mouse model of E. coli-induced peritonitis we found a gradual increase of adenosine levels which peak at 24 hours that gradually declined up to 72 hours from inoculation. The intra-peritoneal influx of leukocytes after inoculation was blocked by the A2AR agonist CGS-21680. In inoculated mice, the A2AR agonist also caused a significant decrease in sera and peritoneal levels of TNF $\alpha$  and IL-6 as compared to untreated mice. By analysis of PMC mRNA and protein levels we found that both CD39 and CD73 levels increased ~3 fold higher than normal at the initial phase of inflammation and decreased at the resolution phase.

These data suggest that upregulation of both CD73 and CD39 in the initial phase of peritonitis is responsible for the increase of adenosine levels, which is a potent regulator of the inflammatory response in the peritoneal cavity.

## Presence of IgG–CD4 complexes in the circulation

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Immunoglobulin (Ig) molecules react with various ligands forming complexes in the circulation as well as on the cell surface [1]. We detected IgG complexes with CD4, an T–cell receptor, in commercial gamma–globulins and in donor sera using anti–CD4 antibodies. It was calculated that in commercial gamma–globulins (Sigma) the molecular ratio between CD4 and IgG was approximately to 1:400. A sensitive dot–blot assay [2] previously effectively employed for the evaluation of antigens in immune complexes [3] was used for detection of IgG–CD4 in donor sera. IgG and their complexes were isolated from sera by Protein G –Sepharose beads. Adsorbed proteins were eluted from beads by acid buffer, pH 2.5 directly on nitrocellulose membrane. Eluted CD4, which immobilized on membrane as small spot, was detected by biotinylated anti–CD4 monoclonal antibodies. IgG–CD4 complexes were found in all studied donor sera. The amount of CD4 in complexes with IgG varied significantly in serum samples from different donors. The presence of CD4 in complexes with IgG in the circulation could be explained by the appearance of free CD4 due to the disintegration of T cells after their death and/or by the process of CD4 shedding from T–cell surface.

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# Lymphocyte Cell Surface Dynamics: The Balance Between Diffusion, Aggregation and Endocytosis

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The cell membrane lies at the interface between an extracellular set of signals and the appropriate intracellular response. Specifically, lymphocyte activity is determined by the spatial and structural response to antigens, as mediated by cell surface receptors. In order to correlate experimentally observed cellular activities, such as secretion, anergy, death, survival and division to external stimuli, it is necessary to monitor cell surface dynamics. B–lymphocyte activation results from the stimulation by large immune complexes comprising antigens, B cell receptors and co–receptors. Compartmentalization of the interacting molecular components is required in order to assure the rapid initiation of specialized and sustained signaling cascades. In this study, a Monte Carlo simulation of the cell membrane dynamics was developed to clarify the receptor dynamics, aggregation mechanisms and their combined effect on cellular functions. This simulation is based on experimentally measured parameters and represents a feasible, advanced and reliable framework to investigate the cell surface. The current study focused on B cell surface dynamics. We developed a model demonstrating the basic properties of B cell receptor (BcR) dynamics and how BcR kinetics is affected by lipid rafts. We studied BcR interactions with multivalent ligands and the influence of lipid rafts on this interaction. Finally, we estimated the dynamics of the initial steps of BcR–mediated cell activation and demonstrated the effect of the association of signaling molecules with lipid rafts. We used these results to suggest some novel hypotheses on BcR–mediated B cell activation.

## **PICOT expression increases in highly proliferating and transformed T lymphocytes**

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PICOT (PKC $\zeta$ -interacting cousin of thioredoxin) is encoded by a novel gene, which was discovered in a search for PKC $\zeta$ -binding proteins in human T lymphocytes (J. Biol. Chem. 275:1902, 2000). It is a relatively abundant protein, which is expressed in a wide range of cell types, predominantly in the cytosol. We found that PICOT expression increases in highly proliferating T lymphocytes in response to mitogenic agents, suggesting its involvement in biochemical pathways that regulate cell growth multiplication. In addition, we found a relatively high level of expression of PICOT in several different human tumor cell lines. To further substantiate our findings, we performed immunohistochemical analyses of tissue sections from human cancer patients that possess different types of tumors. In a lymph node section from a patient with anaplastic large cell lymphoma (ALCL) we found significantly higher levels of PICOT in the tumor cells, when compared to non-transformed lymphocytes within the same tissue. In addition, Reed-Sternberg cells of Hodgkin's lymphoma patients also expressed a relatively high level of PICOT. The increased expression levels of PICOT in lymphoma cells suggest the involvement of PICOT in cell transformation and/or promotion of cell cycle progression. Other studies raise the possibility that upregulation of PICOT correlates with stress conditions. Our current investigations aimed at further dissection of the cellular mechanisms of regulation of PICOT expression, and identification and characterization of the biological roles of PICOT in these cells.

## The Role of Interferon Regulatory factors (IRFs) in Intestinal Inflammation

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The Inflammatory bowel diseases (IBD); Crohn's disease (CD) and ulcerative colitis, are chronic inflammatory disorders of the gastrointestinal tract. These diseases are caused by genetic and environmental factors. Mutations in the Caspase-Activating and Recruitment Domain-15 (CARD15/NOD2) gene, an intracellular repressor of NFkB-dependent Th1 immunity, have been associated with CD. Other candidate effectors of IBD are the Interferon Regulatory Factors (IRFs), a family of transcription factors. IRFs are essential for mounting Th1-mediated immunity and hence affect the inflammatory process. Therefore, they might play a role in intestinal inflammation.

The role of IRFs in the regulation of CARD15/NOD2 gene expression was studied using reporter gene assays in mouse macrophage cell-lines. Overexpression of IRF-4 resulted in dramatic increase of promoter activity (20 fold), while IRF-8 or PU.1 (a hematopoietic essential transcription factor) led to a moderate effect. Promoter deletion analysis indicated that two Interferon Stimulated Response Elements (ISREs), located at the upstream region of the promoter, are essential for IRF-4 activation. Furthermore, ChIP assay indicated that IRFs associate with the CARD15/NOD2 promoter *in vivo*.

We have used a mouse model of chemical-induced colitis to study the role of IRF-8 in IBD. Acute colitis was induced by administering 2% Dextran Sodium Sulfate (DSS) to the drinking water. The severity of the colitis was evaluated in wild type (WT) as well as in IRF-8<sup>-/-</sup> mice using clinical and histological scores. IRF-8<sup>-/-</sup> mice exhibited significantly ameliorated disease compared to WT mice. These data indicate that absence of IRF-8 leads to decreased susceptibility to DSS-induced colitis, supporting the function IRF-8 as pro-inflammatory agent.

## **Cysteamine enhance the antitumoral effect of AS101 in Multiple Myeloma**

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Multiple myeloma (MM) is a plasma–cell neoplasm that is characterized by the monoclonal proliferation of the plasma cells in the bone marrow . It is the second most common haematological malignancy , and accounts for 1% of all cancers. AS101 is a well known organotellurium compound with a variety of antitumoral activities in different tumor models. Cysteamine, which is used as a treatment for cystinosis, is a thiol compound that carry cysteine into the cells. Lately, it was shown in our laboratory that combined treatment of AS101 and Cysteamine induced apoptosis in malignant T cells. In the present study we examined the synergistic effect of the antitumoral compound AS101 with Cysteamine on Multiple Myeloma cell lines. AS101 alone decreased cell proliferation and induced G2/M cell arrest after incubation of 48 hours. Low concentration of AS101 combined with Cysteamine, leaded to those effects after only 20 hours. In addition, early apoptosis was observed after 24 hours. The synergistic effect of AS101 and Cysteamine caused an increase in caspase 3 activity , and also decreased the mitochondrial trans membrane potential following that treatment. Moreover, it was found that the induction of apoptosis and cell arrest by AS101 and Cysteamine lead to down regulation of Bcl–2 and the anti apoptotic protein, survivin ,at different MM cell line. Our results show that Cystemine increase the antitumoral activity of AS101, We assume that Cysteamine sensitize MM cells to AS101 apoptotic ability

## The throughput of the transitional 3 B cell pool accounts for losses between transitional and mature B cells.

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B lymphocytes are subject to specificity based elimination following strong BCR ligation in the absence of appropriate second signals. Substantial immature B cell losses are incurred via this mechanism in bone marrow and periphery, however, mature B cells can also be eliminated through this mechanism, but the phenotypic population containing mature cells destined for specificity based elimination has not been identified. Our study aimed to examine the hypothesis that the transitional 3 (T3) peripheral subset contains cells undergoing negative selection derived from both the emerging and mature B cells pools, and to determine whether this model better explains the dynamics of these pools. To address these issues, we used mathematical models that numerically simulate splenic B cell population dynamics, and fit the models to existing in vivo labeling data to interrogate this new hypothesis. The root mean square deviation (RMS) obtained by fitting the new model to the data was lower than the RMS obtained under the old model. The death rate in T3 B cells was higher than the death rates of all other splenic B cells subpopulations. This result suggests that the new hypothesis explains the observed dynamics of peripheral subsets better than the old model, and that the throughput of the T3 B cells pool can account for most of the losses between transitional and mature B cells, and also contains mature cells that have been induced to undergo selection. Our findings help to understand the process of B cell development in the spleen, which is essential for understanding some of the developmental defects which lead to immune deficiency.

# **A peptide based on the CDR1 of an autoantibody ameliorates lupus manifestations by down regulating apoptosis and the pro-apoptotic factor JNK kinase**

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SLE is an autoimmune disease characterized by autoantibodies, and systemic clinical manifestations. Treatment with a peptide (hCDR1) that is based on the complementarity determining region (CDR) 1 of a monoclonal anti-DNA antibody that bears the common idiotype, 16/6Id, ameliorate disease manifestations of mice with either induced or spontaneous SLE. Aberrant expression and function of the p21Ras/MAP kinase pathway is associated with active SLE. Therefore, we examined the expression and function of the p21Ras pathway and its correlation with apoptosis and disease activity in (NZBxNZW)F1 female mice with preexisting disease that were treated with the hCDR1 peptide. Untreated SLE afflicted mice demonstrated increased expression of p21Ras in conjunction with reduced hSOS and unchanged p120GAP levels, as compared to healthy controls. The expression of these elements was not modified by treatment with hCDR1. However, the late signaling element of p21Ras pathway, JNK kinase, was upregulated, mainly in the T cell population of the diseased mice and treatment with hCDR1 downregulated it. In addition, the T cells of SLE afflicted mice exhibited increased rates of apoptosis mediated via the Fas/FasL pathway. The latter was also diminished following treatment with hCDR1. Importantly, the effects of hCDR1 on both JNK kinase expression and apoptosis were associated with amelioration of the clinical and serological SLE manifestations. Thus, hCDR1 therapy ameliorates SLE, at least in part, via downregulation of the activity of the pro-apoptotic JNK kinase.

## **DOCK-2 is a key regulator of chemokine triggered lateral lymphocyte motility but not transendothelial migration**

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Rac GTPases are key regulators of leukocyte motility in response to both cytokine and adhesive signals. Recent studies suggested involvement of these GTPases in lymphocyte adhesion under shear stress. In lymphocytes, chemokine- and TCR-mediated Rac activation depends on DOCK-2, a member of the CDM adaptor family. Rapid chemokine-triggering of both LFA-1 and VLA-4 integrins took place normally in DOCK-2 <sup>-/-</sup> T lymphocytes under various shear flow conditions. DOCK-2 <sup>-/-</sup> T cells also arrested normally on chemokine bearing TNF $\alpha$ -activated endothelial cells consistent with their normal sticking on HEVs in vivo (Nombela-Arrieta, *Immunity*, 2004). Although DOCK2<sup>-/-</sup> T lymphocytes exhibited reduced microvillar collapse in response to chemokine signals, their resistance to detachment under shear flow was as efficient as of wt T lymphocytes, ruling out a role for this collapse in arrest or subsequent adhesion strengthening. Strikingly, once arrested, DOCK-2 <sup>-/-</sup> lymphocytes efficiently transmigrated through an endothelial barrier presenting CCL21 but could not locomote away from their diapedesis site at the basal endothelial side. DOCK-2 <sup>-/-</sup> lymphocytes also failed to migrate over ICAM-1, VCAM-1 and fibronectin co-immobilized with chemokines as well as on chemokine bearing substrates devoid of integrin ligand. This is a first indication that T lymphocytes use two different chemokine-triggered machineries: the first, DOCK2 and Rac dependent, to locomote laterally on apical and basal endothelial compartments, and the second, DOCK2 independent, to cross the endothelial junction under shear flow.

# **G-CSF enhances the adhesion of encephalitogenic T cells to extracellular matrix components: a possible mechanism for exacerbation of multiple sclerosis. Potential correction by the immunomodulator AS101**

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Autoreactive T-lymphocytes reactive with myelin antigens may be involved in pathogenesis of multiple sclerosis. Extravasation and transmigration across ECM barriers target cells to CNS myelin where they induce inflammation. Chemotherapy followed by autologous stem cell transplantation is considered for treating severe refractory autoimmune disorders including MS. Stem cell mobilization to the periphery is achieved with G-CSF. However, G-CSF administration resulted in several cases of exacerbation of MS and other autoimmune disorders. The mechanism is unclear as G-CSF associates with anti-inflammatory cytokine profile. We examined the hypothesis that G-CSF increases adhesion of autoreactive T cells to ECM proteins, promoting CNS inflammation and contributing to exacerbation of MS. We compared adhesion of MS autoreactive T cell lines to collagen-IV and fibronectin following stimulation with G-CSF in culture to stimulations with IFN $\gamma$  and TNF $\pm$ . In 60% of MS patients, G-CSF enhanced cell adhesion to ECM more effectively than IFN $\gamma$  and TNF $\pm$ , two most notorious pro-inflammatory cytokines known to exacerbate MS. Similar findings were noted with myelin-reactive healthy donor T cell lines. G-CSF thus emerges as most potent inducer of T cell adhesion to ECM, involving adhesion-associated cytoskeletal elements. The increased adhesion mediated by G-CSF indeed induced phosphorylation of the focal adhesion protein, (Pyk2 and its downstream signaling targets ERK 1/2. Herein we offer means of counteracting G-CSF effects using the tellurate immunomodulator AS101. AS101 inhibited G-CSF and IFN $\gamma$ -induced T-cell adhesion and may be beneficial therapeutically in inflammatory diseases.

## **Insights into the regulation of the pro-malignancy chemokine CCL2 and its activities in breast cancer**

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Tumor development and progression are multifactorial processes, regulated by a large variety of factors. Specifically in breast carcinoma, the chemokines CCL2 and CCL5 are associated with elevated malignancy.

Our studies indicate that in human breast carcinoma cells, MCF-7 and T47D, CCL2 is constitutively secreted and stored in intracellular stores. To determine the role of de novo protein synthesis in CCL2 production, the cells were treated with the protein synthesis inhibitor cycloheximide (CHX). The results indicate that CHX caused an increase in the basal level of CCL2, indicating that the constitutive expression of the chemokine is regulated by mechanisms that require protein synthesis. In addition, CCL2 was found to be regulated at the protein stability level: treatment of the cells by lactacystine, a specific inhibitor of the proteosomal pathway of degradation led to an increase in extra cellular levels of CCL2. Furthermore, CCL2 was found to be regulated by dipeptidyl peptidase IV (CD26), which is known to degrade many chemokines, as its inhibition led to a decrease in the secretion of CCL2 by the tumor cells.

Further, our studies indicate that CCL2 potently promotes the extracellular expression of CCL5 in human breast carcinoma cells. Determination of the signaling mechanisms revealed that Erk and PI3K are involved in this process. Furthermore, Erk and PI3K were found to regulate the basal levels of CCL5 expression, suggesting that autocrine mechanisms control CCL5 expression.

Overall, our findings provide insight into mechanisms that are involved in the regulation and activities of CCL2 in breast tumor cells

## **Regulation of inducible heparanase gene expression in human T-cells by soluble and immobilized TNF-alpha**

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In the course of an inflammatory response, immune cells acquire a directional migrating phenotype. This is done by degradation of the extracellular mesh with enzymes and by promoting adhesion and migration of the cells by chemokines and cytokines. One of the enzymes is heparanase, a heparan sulfate specific endoglycosidase. Here we examined the regulation of heparanase expression by cytokines in human CD3+ T-cells from healthy donors. Both soluble and fibronectin-immobilized TNF-alpha promoted the accumulation of heparanase mRNA and protein in a time- and concentration-dependent manner. Heparanase mRNA accumulation depended on PKC, JNK and PI3K signaling, but not on NFkB. The regulatory effects of TNF-alpha on heparanase expression were accompanied by up-regulation of relevant transcription factors such as GATA-3, Egr-1, Ets-1, but not that of Ets-2. These effects were partially reproduced by TNF superfamily members, FasL and TRAIL. The expression of heparanase by T-cells is thus tightly regulated by inflammatory cytokines.

## **Cell Surface CD74 initiates a signaling cascade leading to cell proliferation and survival**

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Differentiation of immature into mature B cells is essential for B cell responsiveness in the humoral immune response. CD74 (invariant chain) is a non-polymorphic type II integral membrane protein that was thought to function mainly as an MHC class II chaperone. However, CD74 was recently shown to have an additional role as an accessory signaling molecule. We have previously shown that CD74 is cleaved and its intracellular fragment (CD74-ICD) is liberated from the membrane. This fragment enters the nucleus and induces a pathway leading to the activation of transcription mediated by the NF- $\kappa$ B p65/RelA homodimer and its co-activator, TAFII105. To follow cell surface CD74 function, B cells or CD74 293 transfected cells were activated with anti-CD74 antibody, and its downstream signaling cascade was analyzed. Here we show that CD74 stimulation leads to Syk tyrosine kinase and the PI3K/Akt phosphorylation resulting in NF- $\kappa$ B activation. This pathway leads to the entry of the stimulated cells into the S phase, elevation of DNA synthesis, cell division, and augmented expression of BCL-XL. These studies therefore demonstrate that surface CD74 functions as a survival receptor.

## **IGV Lineage Tree Analysis of B lymphocytes found in the CSF and PBL of MS Patients.**

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Multiple Sclerosis (MS) is a chronic inflammatory demyelinating disease of the central nervous system (CNS) in which infiltrating mononuclear cells, lead to the damage of the myelin sheath. While the main focus of MS studies is centered on the role of the T cells in the initiation of the inflammatory symptoms, auto–antibodies produced by B cells are also observed to be involved in the destruction of myelin in the course of the disease. Moreover, studies found that B lymphocyte clones present in the CSF of MS patients undergo intraclonal diversification. In order to study the dynamics of intraclonal expansion of B lymphocytes in MS, lineage trees were used to investigate published IGV sequences from B cell clones extracted from the CSF and PBL of MS patients. In order to acquire more information from the IGV sequences, a novel mathematical method for analyzing the graphical properties of IGV gene lineage trees was used. B lymphocyte lineage trees created from the MS patients were compared to trees from normal controls. We found that MS patient B cell clones had a high rate of diversification, as their trees were larger than those of normal controls, as is expected in a chronic reaction. Nevertheless, the B lymphocyte selection process appeared to remain intact. A second analysis performed, compared IGV lineage trees from two MS patients at two time intervals within the disease, and found that no significant changes existed between the tree groups from the two time intervals, although, over time, a decrease in diversification was seen to occur in both patients while the selection pressure remained unchanged.

# The In Vivo Differentiation Potential and Interrelation of Mononuclear Phagocyte Precursors

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The Mononuclear Phagocyte (MP) system of higher vertebrates is a heterogeneous network of myeloid cells spread throughout the organism. Distinct phenotypes and segregated anatomic location of its main two representatives, e.g. macrophages (MF) and dendritic cells (DC) suggest differential contribution to appreciated MP functions in homeostasis, inflammation and immune defense, which could potentially be exploited for therapeutic manipulations. In depth insight into in vivo task division of MP is however hampered by the fact that in vivo differentiation of MP remains poorly understood. Here we use adoptive precursor transfer experiments to establish a complete sequence of myeloid in vivo differentiation beginning with a novel bone marrow (BM) precursor, termed MDP, that has the unique potential to differentiate into MF and DC, but not granulocytes (Fogg et al, Science 2006). Intra Bone Cavity Transfer of these MDP into wt mice yielded BM and blood monocyte intermediates. Furthermore, MDP descendants efficiently seeded both lymphoid and non-lymphoid organs, such as the intestinal lamina propria with terminally differentiated MF and DC. Highlighting the dynamics of precursor cell migration, we show that a recently reported Gr1+ "inflammatory" blood monocyte subset (Geissmann et al. Immunity 2003) shuttles in the absence of inflammation efficiently back to the BM, where it is re-cycled and contributes to the generation of peripheral BM resident MPs.

## Evaluation of acupuncture of sjogren's patients on clinical and laboratory and laboratory findings

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Sjogren's syndrome is a systemic autoimmune disorder, characterized by salivary and lacrimal gland inflammation. This study was carried out on twenty (20) Sjogren patients. Acupuncture treatment was administered twice a week for a period of six weeks, followed by six additional weeks of acupuncture treatments once a week. Patients were randomly divided into two groups. Placebo treatment was administered at the same frequency and duration as the treated group. The Evaluating parameters were measured from base line to 3 months preceding the completion of treatment. Data analysis was performed by using t-test. Significance was assigned to values of  $p < 0.05$ . Results: 1. Acupuncture, in contrast to placebo, improved eye damage and saliva quantity 2. Both treated and placebo group reported an improvement of symptoms (subjective reports). However, only the treated group's reports were correlated with the clinical symptoms. 3. Placebo treatment changed values of IgM, ESR, C3, C4, C7, C1- Inh, and Properdin. Acupuncture treatment changed values of IgM, IgG, a1 globulin, C3, and C6 4. Complement CH50 revealed no significant difference between the two groups. Conclusions:

1. Preliminary results indicate that the decrease of various complement components, due to the inflammatory process, was halted by acupuncture treatments.
2. Acupuncture treatments influence the quantity over the activity of the complement mechanism.
3. Clinicians should be wary of evaluating patients solely by questionnaires.
4. The effect of acupuncture on Sjogren's disease is significant, and should be considered as an adjuvant treatment to conventional care.

## Heat Shock Protein 60 is an Innate Enhancer of CD4+CD25+ Regulatory T-Cell Activation and Function

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CD4+CD25+ T regulatory cells (Tregs) regulate immunity, but little is known about their own regulation. We now report that the human 60 kDa heat shock protein (HSP60), administered before mitogenic anti-CD3 activation, acts as a co-stimulator of human Tregs. CD4+CD25- or CD8+ T cells did not respond innately to HSP60, but treatment of relatively low numbers of Tregs with HSP60, or its peptide p277, significantly enhanced their down-regulation of CD4+CD25- or CD8+ target T cells, detected as inhibition of target T-cell proliferation and IFN- $\gamma$  and TNF- $\alpha$  secretion. The enhancing effects of HSP60 on Tregs involved innate signaling via TLR-2, led to activation of PKC, PI-3 kinase, and p38, and were further enhanced by inhibiting ERK. Limited numbers of HSP60-treated Tregs suppressed target T cells both by cell-to-cell contact and by secretion of TGF- $\beta$  and IL-10. The down-regulated target T cells manifested inhibited ERK, NF- $\kappa$ B and T-bet. Thus, HSP60 can down-regulate adaptive immune responses by up-regulating Tregs innately.

## **CXCL10 as a potential pro-malignancy factor in colorectal carcinoma**

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Metastasis is the major cause of death of patients with colorectal carcinoma (CRC). Liver, lung and lymph nodes are common sites of CRC metastases. Chemokines play prominent roles in cell migration and growth, and thus may regulate haptotaxis and metastasis formation of CRC.

The aim of the present research was to identify the roles played by chemokines present at metastatic sites of CRC, and by their corresponding receptors expressed by CRC cells, on growth and metastasis. RT-PCR analysis indicated that the chemokine CXCL10 is present in CRC metastatic sites, and that CRC tumor cells express 3 variants of its corresponding receptor, CXCR3. CXCR3 expression by these cells was also detected at the protein level.

CXCL10 was thus far characterized as an anti-cancer factor acting on cells of the tumor microenvironment. In contrast, our findings suggest that the chemokine affects directly CRC cells and may promote their malignancy phenotype. Specifically, the results indicate that CXCL10 up-regulates the expression of the 64 kDa MMP9 and the 95 kDa MMP9, and induces the migration of CRC cells. These results suggest that CXCL10 promotes CRC motility and invasion, and imply that CXCL10 is a pro-malignancy factor in CRC. Moreover, the exposure of CRC cells to the cytokine interferon  $\gamma$ , currently considered as a potential anti-malignancy factor, gave rise to highly potent elevation in CXCL10 release by CRC cells.

In view of the above, we suggest that the consideration of CXCL10 as an anti-malignancy factor in CRC should be carefully studied.

# Lineage tree analysis and the dynamics of FL and DLBCL

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Much information about the dynamics of hypermutation and antigen-driven clonal selection during the humoral immune response is contained in the shape of mutational lineage trees deduced from the responding clones. We have designed a novel algorithm for quantifying, in terms based on graph theory, the shape properties of mutational lineage trees. This method can reveal fine details about the clonal histories and the balance between diversification and antigen-driven selection. In this study we have applied this algorithm to lineage trees from follicular lymphoma (FL) and diffuse large B cell lymphoma (DLBCL), which are B cell malignancies of a germinal center origin. Lineage tree analyses demonstrates that FL and DLBCL clones have a higher intraclonal diversity than normal B cell clones, that their diversification processes have been ongoing for a longer time and that their responses to selection pressures are impaired in comparison to the normal controls.



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