Instituto Juan March
de Estudios e Investigaciones

54 CENTRO DE REUNIONES
INTERNACIONALES SOBRE BIOLOGÍA

Workshop on

Cytokines in Infectious Diseases

Organized by

A. Sher, M. Fresno and L. Rivas

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INTRODUCTION

M. Fresno and A. Sher

In recent years it has become clear that cytokines play an important role in controlling both the inductive and the effector arm of the immune response. Moreover, the host cytokines response is a key determinant of the outcome of infection-governing both host resistance and immunopathology. For this reason, prophylaxis or treatment with cytokines has emerged as an important strategy for immunologic intervention in many infectious diseases.

In addition, parasite infections provide paradigms for addressing some fundamental questions concerning the innate host response to infection. What parasite molecules stimulate cytokine production? What causes in some cases, the abnormally high cytokine production that lead to severe pathology? Is this genetically regulated? The study of cytokine function in infectious disease has been revolutionized by the advent of engineered mouse strains with genetic disruptions in cytokine and cytokine receptor genes. In addition to identifying cytokine requirements for host resistance and pathology, these animals have provided new insights into redundancies in the cytokine network itself. Such investigations have highlighted the important lessons learned from the infection disease models on the mechanism underlying the selective induction of different immune responses.

There are two main types of helper T (Th) cells according to cytokine secretion. Th1 cells produce IL-2, INF-γ, and TNF but not IL-4. By the contrary, Th2 cells preferentially secrete IL-4, but not no IFN-γ. Their polarized expression in different disease states frequently determines host resistance or susceptibility and it is strongly influenced by events triggered early in infection, involving innate recognition mechanisms. The early induction of IL-12 by APCs, which in turn trigger IFN-γ, is a key determinant of Th1 response induction while the initiation of Th2 responses depends on IL-4. Thus, the balance of IL-12 and IL-4 triggered early after pathogen invasion forms the basis of the subsequent selection of T cell subsets and their protective versus disease promoting influence on infection. In many diseases, such as Leishmaniasis, Toxoplasmosis, Tuberculosis, Th1 responses are protective whereas Th2 are detrimental. Others, as Chagas or Malaria have a more complex pattern.

Th cell polarization is a complicated process controlled by a number of factors including: the nature of the antigen and of the APCs, accessory molecules expressed on APCs that deliver different co-activation signals to T cells, cytokines produced early after exposure to a pathogenic agent or immunization with and antigen, etc. There is now growing evidence that cells other than APC, encountered

by pathogens early after host entry, such as neutrophils and epithelial cells are also capable of producing IL-12. Besides, $CD4^+NK1.1^+$ cells, and in the case of the *Leishmania* model, a subset of $CD4^+$ cells with a limited TCR repertoire, have been implicated as sources of the IL-4 in addition to T and mast cells.

In addition to their role in initiating T cell subset differentiation, cytokines are crucial for maintaining and regulating adaptive immune responses. The lymphokines IL-2, IL-4, IFN-γ and the anti-inflammatory cytokines IL-10 and TGB-β are key players at this stage. An important effector mechanism involved in the control of many different infectious agents is the production of nitric oxide (NO). The synthesis of this toxic metabolite is induced by the action of the Th1 cytokines and regulated by both Th2 and anti-inflammatory cytokines.

Pathogens have also evolved complex strategies to ensure survival in an immunologically hostile host environment. Thus, many parasites have coevolved molecules that can alter the production of either immunoregulatory or effector cytokines, important to control the infection, by macrophages.

Although clearly important in both the establishment and maintenance of resistance, the cytokine response to infectious agents can also be host detrimental and has been described as a "double-edged sword". Most of the pro-inflammatory cytokines and lymphokines associated with the Th1 response (e.g. TNF-α, IL-12 and IFN-γ) are toxic when induced in an excessive or uncontrolled manner. Some of this toxicity results from the subsequent production of NO but also from more complex down-stream phenomena. Cytokine biology offers an important approach for understanding the pathogenesis of these disorders. Finally, as should be obvious, the exogenous manipulation of deleterious cytokine responses offers a potentially powerful strategy for preventing or treating infectious disease pathology.

Animal studies on a number of important infectious diseases have provided testable strategies for the use of cytokines in disease treatment as well as prevention. The further elucidation of the function role of cytokine regulation in human infection and the continued introduction into the clinic of novel cytokine based strategies for disease intervention remain important goals for this field. The Juan March Workshop has provided a forum to address some of those questions in detail and to exchange knowledge from different infectious diseases in this dynamic field.

Session I: Cytokine regulation of CD4+ subset differentiation: The *Leishmania* paradigm

Chairman: Jacques A. Louis

Attempts to decipher *in vivo* the early events directing the development of functionally polarized CD4+ T cell responses in mice infected with *Leishmania major*

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The murine model of infection with *Leishmania major* provided the first clear correlation between the development of protective immunity and the expansion of Th1 cells and progressive disease and the expansion of Th2 CD4+ T cells. Furthermore, differentiated *L. major*, specific Th1 and Th2 cells have been demonstrated to mediate, respectively, resistance and susceptibility to infection with this parasite. Thus, the murine model of infection with *L. major* has been used by several laboratories for dissecting the mechanisms controlling the differentiation of CD4+ T cell subsets *in vivo*.

Inasmuch as evidence, albeit indirect, demonstrates that IL-4 has an important role during the initial stage of infection in the subsequent development of specific CD4+ Th2 response in susceptible BALB/c mice, we have compared the IL-4 gene expression during the early stages of infection in susceptible and resistant C57Bl/6 mice. In contrast to resistant mice, susceptible BALB/c mice exhibited a peak of IL-4 mRNA expression in draining lymph nodes as soon as 16 hrs after infection with *L. major*. Following this very rapid burst, a sharp decline of IL-4 mRNA expression occured 24 hrs after infection returning to control levels of uninfected mice by 48 hrs. At 4 to 5 days, however, increase of IL-4 mRNA was again seen and remained stable for, at least, up to 10 days. The early (16 hrs) peak of IL-4 mRNA expression was never seen in C57Bl/6 mice. At day 4, a small increase in IL-4 mRNA 5 fold lower than in BALB/c mice was observed in these mice returning to base line levels by day 7.

Results will be presented illustrating the importance of the early (16 hrs) IL-4 response to *L. major* to the subsequent development of polarized Th2 response in BALB/c mice. Interestingly, this very rapid burst of IL-4 production in BALB/c mice was abrogated by IL-12 and/or IFN-γ administered before parasite inoculation. Reciprocally, neutralization of endogenous IL-12 and/or IFN-γ allowed the expression of this early peak of IL-4 mRNA in resistant mice. Thus, these results support the notion that the effect of IL-12 on Th1 cells development could be, at least in part, the result of the ability of this cytokine to downregulate the initial IL-4 production required for Th2 cell differentiation. Furthermore, results of recent experiments, will be presented, showing that the IL-4 produced early in BALB/c mice in response to *L. major* renders rapidly T cell precursors unresponsive to the Th1 differentiating effect of IL-12.

Interestingly, the NK1.1+ CD4+ T cells, demonstrated to produce IL-4 rapidly following injection of anti-CD3 mAb, did not contribute to the rapid IL-4 response triggered by *L. major* in either BALB/c mice or resistant mice provided that these mice are treated with anti-IL-12 prior to infection. The rapide IL-4 response to *L. major* is the results of the activity of CD4+ NK1.1- T cells that may express a limited TCR repertoire.

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Probing the Biology of Murine Leishmaniasis with T Cell Receptor Transgenic Mice

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Infection of inbred strains of mice with *Leishmania major* has defined an exceptionally characterized model of CD4+ effector subset development (1). Most mice develop self-limited, localized lesions at the site of inoculation that heal coincident with the development of Th1 effector cells capable of supplying IFN-γ that is required for activation of macrophages, the only host cell productively infected by these organisms. In contrast, mice on a BALB background, including BALB/c, BALB.B and BALB.K mice, aberrantly develop parasite-specific Th2 effector cells that abrogate the capacity of Th1 cells to mediate cure. The use of neutralizing antibodies and knock-out animals has defined critical roles for IFN-γ and IL-12 in effective Th1 development and for IL-4 in Th2 development. Despite these insights, the genetic basis for aberrant Th2 cell development in BALB mice remains undefined.

We began studies of the CD4+ T cell response in recognition of the capacity of these cells alone to reconstitute the spectrum of infection in SCID mice (2) and with the observation that MHC class II null mice (3), but not MHC class I null mice (4), were unable to heal infection. Such studies identified early expansion of CD4+ T cells that utilized Vβ4/Vα8 T cell receptors (TCR) (5). Sequencing of cloned T cells that used this receptor demonstrated conservation of CDR3 length and charge, consistent with a response to a single immunodominant epitope from a parasite antigen. Cloned T cells were used to identify the antigen as LACK, a conserved *Leishmania* homolog of RACK1, the receptor for activated C kinase (6). Clones were used to map the epitope of LACK to a single 18 amino acid sequence in the fourth W-D domain of LACK. Immunization of BALB/c mice with full-length recombinant LACK consistently generated CD4+ T cells in the draining lymph nodes that expressed Vβ4/Vα8 TCR and reacted with the dominant 18 amino acid peptide. Direct binding assays confirmed the high binding affinity of the peptide for I-A^d and confirmed the crucial role of a charged histidine residue in T cell activation.

Genomic sequences of rearranged Vβ4 and Vα8 genes from a LACK-reactive clone were used to generate TCR transgenic mice. The mice were backcrossed to both the BALB/c and B10.D2 backgrounds and infected with *Leishmania major*. The B10.D2 TCR mice healed infection and demonstrated earlier and more exuberant IFN-γ

production in response to LACK. Thus T cell reactivity to this single dominant antigen was sufficient to mediate healing of this complex parasitic infection. In contrast, BALB/c TCR mice developed large lesions early, but, unlike TCR-negative littermates, ultimately began to contain the infection and demonstrated strong Th1 responses. Analysis of the TCR mice revealed that the transgenic T cells were largely CD4-CD8-, in contrast to the CD4 expression on the donor clones. Crossing the mice to the rag null background revealed a peripheral repertoire of 95% double negative cells and 5% CD4+ T cells. Both the double-negative and the CD4+ cells were class II-restricted and LACK-reactive. Surprisingly, however, the double-negative cells were neither able to generate IL-4 in vitro nor be conditioned to become Th2 cells in vitro by repeated stimulation in the presence of IL-4. In contrast, the CD4+ T cells on the BALB/c background readily generated IL-4 in vitro that was much greater than the same TCR transgenic T cells on the B10.D2 background. These results suggest that the strength of signal generated during T cell-APC interaction may profoundly effect the capacity to generate effector Th2 cells. This was corroborated by infection of CD4 null mice, which can readily generate class II-restricted Th1 cells that mediate cure against *Leishmania major* (3), with *Nippostrongylus*. These mice were unable to expulse worms and failed to generate IL-4-producing cells or systemic IgE.

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The Role of IL-12 in the Regulation of CD4+ T helper cell subsets in experimental leishmaniasis. Phillip Scott, Department of Pathobiology, School of Veterinary Medicine, University of Pennsylvania, 3800 Spruce Street, Philadelphia, PA 19104, USA

L. major infection in C3H mice is associated with healing, an early NK cell response, and the early development of a CD4+ Th1 cell response, while BALB/c mice fail to exhibit an NK cell response, develop a Th2 response, and fail to control the parasite (1). Since IL-12 plays a central role in NK cell activation and the development of CD4+ Th1 cells in vitro, we investigated what role IL-12 might play in: (1) the development of resistance to *L. major* in C3H mice; (2) the initiation of a protective Th1 response in a leishmanial vaccine; and, (3) modifying an established Th2 response in *L. major* infected BALB/c mice. The association that IL-12 has with the development of Th1 cells would suggest that in resistant mouse strains, infection with *L. major* should induce an IL-12 response. Indeed, IL-12 levels and the number of cells producing IL-12 increased in the lymph nodes draining the infection site of C3H mice at 1 to 2 days, corresponding to the peak NK cell response that we previously observed in these mice (1,2). While this data might be expected, there has been some confusion regarding the role that IL-12 plays in leishmanial infection. This has stemmed partly from the observation that *L. major* promastigotes fail to stimulate IL-12 production by purified macrophages in vitro (3). However, we found that following intraperitoneal inoculation, *L. major* induced IL-12 production by peritoneal cells (4). The differences in the in vitro and in vivo systems remain unexplained. Nevertheless, the in vivo data clearly demonstrates that IL-12 is induced following infection with *L. major*. To further demonstrate the importance of IL-12, C3H mice were treated with monoclonal anti-IL-12 antibody. We found that antibody neutralization of IL-12 ablated the early NK and IFN-γ response in these mice, indicating that the IL-12 observed was required for this response (2). Anti-IL-12 treated C3H mice also failed to develop a Th1 response, and developed much larger lesions than control mice.

In order to take advantage of the ability of IL-12 to initiate Th1 cell development, we incorporated IL-12 as an adjuvant into a leishmanial vaccine. We found that BALB/c mice immunized with soluble leishmanial antigen (SLA) alone were not protected against a fatal *L. major* infection, while mice immunized subcutaneously with SLA and IL-12 were completely protected against disease (5). Similar to the natural infection in C3H mice, IL-12 functioned by initiating a leishmanial-specific Th1 response, which was dependent upon early induction of NK cells and IFN-γ production. Taken together, our data suggest that IL-12 is a critical cytokine for the in vivo development of CD4+ Th1 cells, and that inclusion of IL-12 may simplify the development of vaccines against diseases controlled by cell-mediated immunity.

We next investigated whether IL-12 could be used as part of an immunotherapy or therapeutic vaccine. In our initial experiments we administered IL-12 systemically after *L. major* infected BALB/c mice had developed a Th2 type response. We found that treatment of BALB/c mice 3 weeks after *L. major* infection with IL-12 alone, administered by a variety of routes, significantly delayed lesion development, but could not promote healing. Such treatment did not lead to any permanent change in the dominance of the Th2 cell population in mice. In order to determine if the presence of large numbers of parasites in the 3 week infected mice inhibited the ability of IL-12 to expand a Th1 population in vivo, we administered the antimony-based leishmanicidal drug Pentostam. In BALB/c mice treated with Pentostam there was delayed lesion development, although once treatment was suspended, the mice developed an uncontrolled infection. In contrast, when mice were treated with both IL-12 and Pentostam they healed their lesions, and associated with this therapeutic effect was a switch from a dominant Th2 to a Th1 type of immune response (6). Moreover, when IL-12 and Pentostam treated mice had healed they were rechallenged with *L. major*, and were found to be resistant. These data indicate that IL-12 can permanently alter the dominant Th cell phenotype from a Th2 to a Th1 type.

The ability of IL-12 to switch from a Th2 to a Th1 phenotype in *L. major* infected BALB/c

mice when the animals were treated with Pentostam could be due to a decrease in parasite load, or a transient increase in antigen load due to the leishmanicidal effects of the drug. In order to differentiate between these two possibilities, cells from 3 week infected mice were transferred to scid mice, which were then simultaneously infected with *L. major* and treated with IL-12. While scid mice receiving cells from infected mice rapidly developed lesions, mice that received cells and IL-12 controlled the infection (7). These data suggest that IL-12 can influence a Th2 cell population in a low antigen environment, suggesting that the efficacy of IL-12 and Pentostam in BALB/c mice was related to decreased parasite load.

Applying our new knowledge of the factors that control CD4+ Th cell differentiation to vaccine development will be critical for the next generation of vaccines. It is no longer sufficient for a vaccine to induce an immune response. Rather, the vaccine must induce the appropriate immune response for the pathogen. IL-12 has been shown to be one of the major initiators of cell-mediated immunity through its ability to enhance Th1 cell differentiation from naive cells (8). We now show that for the development of vaccines requiring cell-mediated immunity, IL-12 or substances that induce IL-12, will provide the necessary signals to insure that the appropriate immune response is generated. In addition, IL-12 may also be important for designing therapeutic vaccines to treat non-healing cases of human leishmaniasis, as well as other infectious diseases where a dominant Th1 response is required for resistance.

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THE EARLY IL-4 RESPONSE TO INFECTION WITH *L. MAJOR* CHARACTERISTIC OF SUSCEPTIBLE BALB/c MICE RAPIDLY INDUCES UNRESPONSIVENESS TO IL-12; *IN VIVO* AND *IN VITRO* EVIDENCES

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There is strong but indirect evidence that Interleukin-4 (IL-4) plays an important role in directing the $CD4^+$ Th2 type response in susceptible BALB/c mice following *L. major* infection. Thus, we compared the expression of IL-4 in draining lymph nodes of susceptible and resistant mice during the first days after infection of *L. major*. In susceptible mice, an early peak in IL-4 mRNA expression was observed in the lymph nodes as soon as 16 hrs following subcutaneous (s.c.) injection of parasites. After this initial burst, there was a sharp decline in IL-4 mRNA expression. At day 4, higher levels of IL-4 mRNA were again detected in LN of susceptible mice possibly reflecting the differentiation of Th2 $CD4^+$ T cells. The early peak in IL-4 mRNA was not observed in resistant mice.

Injection of IL-12 at the time of parasite injection results in the inhibition of both peaks of IL-4. This effect of IL-12 was IFN-γ dependent as injection of anti-IFN-γ into IL-12 treated mice inhibited the suppressive effect of IL-12. In resistant mice, treatment with anti-IFN-γ or anti-IL-12 allowed expression of this early peak of increased IL-4 mRNA levels. Thus IL-12 and IFN-γ are important in regulating this initial burst of IL-4.

There is recent experimental evidence that this rapid IL-4 expression (16hrs) is important for the subsequent development of a Th2 response by susceptible mice.

We have previously shown that the $NK1.1^+$ $CD4^+$ cells, previously demonstrated to produce IL-4 following injection of anti-CD3 mAb, do not contribute to the early peak of IL-4 in response to *L. major* which is produced by $NK\ 1.1^-$ $CD4^+$ T cells. Work is in progress to further define the characterization of the cells contributing to this IL-4 burst. Preliminary results strongly suggest that the TCR repertoire of $NK\ 1.1^-$ $CD4^+$ T cells responsible for this early IL-4 response to *L. major* is limited. Further experiments to determine the activation stage, i.e. naive or memory, of these cells are underway.

ROLE OF CYTOTOXIC T LYMPHOCYTES AND IFN-g IN EXPERIMENTAL CANINE VISCERAL LEISHMANIASIS.

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Evidence of the role of cytotoxic T lymphocytes as one of the effector mechanisms in protection against protozoan parasites has recently been accumulated. Although, cytokines such as interferon-gamma (IFN-g), produced by the Th1 subset has been shown to play a major role against Leishmania infection, evidence for other protozoan parasites such as T. gondi and Plasmodium spp., shows that the protective capability of T-cells may also be due to their cytolityc activity.
We have generated Leishmania-specific T-cell lines from peripheral blood of experimentally infected asymptomatic dogs, that express cytolitic activity against *Leishmania infantum* infected target cells.
Specific lysis by this T-cell lines could be induced after stimulation with irradiated autologous peripheral blood mononuclear cells as antigen presenting cells and parasite soluble antigen. The cytotoxic effector cells generated, lyse infected autologous, but not allogenic target cells, indicating that the cytotoxicity measured in this systen is MHC-restricted. We also observed that these T-cell lines produce IFN-g following antigen stimulation. These results suggests that, in addition to the protective role of cytokines such as IFN-g, cytotoxic T lymphocytes may also play an important role in protection against this parasitic infection.

JUAN ANGUITA

IL-12 RECONSTITUTES MURINE LYME DISEASE CAUSED BY NON-PATHOGENIC BORRELIA BURGDORFERI

Costimulation mediated by B7-1 and B7-2 and their counterparts on T cells (CD28 and CTLA4) has been implicated in the differential development of T cell helper (Th) responses (Th1 vs Th2). We assessed the role of these costimulatory signals on the course of murine Lyme borreliosis because experimental Lyme arthritis is reportedly dependent upon the development of a predominant Th1 response. Treatment with an anti-B7-2 mAb produced a modest increase in arthritis severity in 6 week old C3H/HeN mice, whereas the administration of an anti-B7-1 antibody had no significant effect on arthritis. No differences in disease were found in anti-B7-1 and/or B7-2 mAb treated, 3 week old mice. $CD4^+$ T cells from treated and untreated infected 6 week old mice had similar in vitro proliferative responses to *Borrelia burgdorferi*, and IFN-γ levels were lowest and IL-4 levels were elevated in the anti-B7-1 mAb treated mice. INF-γ levels were also reduced in the sera of the anti-B7-1 and/or B7-2 treated mice. In addition, anti-B7-1 mAb treated mice had elevated *B. burgdorferi*-IgG titers during *B. burgdorferi* infection. These results suggest a role for B7-1 and B7-2 mediated costimulation in modulating the immune response during *B. burgdorferi* infection, and show that signaling delivered by B7-2 may play a partial role in determining the severity of acute murine Lyme arthritis.

GM-CSF mediates protective immunity against HSV-1 encephalitis through CD3$^+$ T cells

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Models of herpes simplex virus type 1 (HSV-1) infection was developed in F344 rats to study systemic immune responses established through various locations of viral inoculation. Following intravitreal (ivt) HSV-1 injection, animals developed a distinct colony-stimulating factor (CSF)-producing T cell population, characterized by serum CSF production and associated granulo-monocytosis, that was induced by following intraperitoneal viral challenge. However, neither intraperitoneal (ip) infectious nor intravitreal UV-inactivated HSV-1 priming established the T cell population in rats. When RT-PCR was conducted on cytoplasmic RNAs purified from splenic CD3$^+$-T cells, only ivt-primed lymphocytes expressed ganulocyte-macrophage (GM)-CSF mRNA after coculture with HSV-1, but neither G-CSF, IL-3, nor IL-6 mRNAs were being expressed. T cells obtained from both ip-infectious virus- and ivt-UV-inactivated virus-primed rats failed to express GM-CSF mRNA. Serum CSF production associated with granulo-monocytosis was again observed in homologous nude rats after adoptive transfer of ivt-primed CD3$^+$ -T cells together with concomitant ip-challenge. To evaluate the effect of GM-CSF production on anti-viral immunity, HSV-1-primed, ip-challenged rats received lethal ocular challenge of the virus. Only GM-CSF- producing, ivt-primed rats were protected ($p<0.0015$). The unique anti-HSV-1 immunity mediated by GM-CSF was further supported by the experiments in which F344 rats pretreated with recombinant murine GM-CSF were protected against lethal encephalitis induced by the ocular challenge of the virus ($p<0.01$). The efficacy of GM-CSF production for anti-viral immunity will be discussed in relation to host defense mechanisms against HSV-1 infection in the central nervous system.

Session II: Lessons from studies on knock-out mice

Chairman: Werner Müller

Cytokine deficient mouse mutants generated by gene targeting

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Homologous recombination in murine embryonic stem cells allows introduction of defined mutations into the mouse germline. We have used this technology to generate mouse mutants deficient for individual or multiple cytokines. For example, we obtained mouse mutants deficient for IL–4, IL–2 and IL–4 or deficient for IL–2, –4,–7, –9, –15. In these mutants the deficiency is present in the animal throughout life and the immune system can compensate for the deficiency. In order to overcome this limitation of the system, we have recently utilised the cre/loxP system to remove cytokine receptors in vivo either cell type specific and/or at a given time point. Using this system we have generated mouse mutants deficient for either the common gamma chain, disrupting the action of IL–2, –4,–7, –9, –15, or for gp130, inactivating IL-6, CNTF, CNTF2, CT-1, IL–11, LIF and OM.

T helper subset development and effector responses in mice deficient for either IL-5, IL-4, IFN-γ R1, or doubly deficient for IL-4/IFN-γ R1.

Manfred Kopf, Marijke Barner, Werner Solbach, Frank Brombacher

We have used mice deficient for cytokines (e.g. IFN-γ, IL-4) that are believed to be critical for the differential regulation of post-thymic CD4+ T cell development into Th1 and Th2 subset and, moreover, for the regulation of effector responses such as macrophage activation and eosinophilia. The mechanism of T helper subset development and effector responses in the various deficient mice (listed in the titel) with several models including repetitive *in vitro* anti-CD3 stimulation of purified resting CD4+ T cells of the various deficient mice and infections that polarize CD4+ responses into type 1 (e.g. *Listeria monocytogenes*) into type 2 (e.g. *Nippostrongylus brasiliensis*) and into type 1 or 2, dependent on the genetic background (e.g. *Leishmania major*). These studies showed that the presence of IFN-γ R1 is not required for Th1 development but crucial for macrophage activation. IFN-γ R1 deficient mice succumb to infections with low titers of *L. major* and *L. monocytogens* because they fail to activate macrophages. Infection with *L. monocytogenes* in these mice is characterized by an extensive neutrophilia, that is responsible for a variety of inflammatory cytokines including IL-12. Normal levels of IL-12 in IFN-γ R1 deficient mice appears to quarantee Th1 development. IL-4 seems to play little if any role for Th1 responses after infection with *L. monocytogenes* or *L. major* on a resistant background (129Sv). To the latter, mice (129Sv) doubly deficient for IL-4/IFN-γ R1 are similarly susceptible to *L. major* as compared to mice (129Sv) deficient for IFN-γ R1 alone.

Th2 development in IL-4 deficient mice, so far, has only been studied by measurement of surrogate Th2 cytokines (e.g. IL-5, IL-10) and type 2 effector responses (e.g. eosinophilia). In IL-4 deficient mice IL-5 production and eosionophila are impaired but not absent suggesting that in vivo another cytokine has a redundant activity for the development of Th2 cells. Interestingly, we found that the residual IL-5 production and eosinophilia in IL-4 deficient mice was dependent on the gentetic background, in addition to IL-4. IL-5 production and eosinophilia was strongly reduced in IL-4-deficient mice on the 129Sv background but only very moderately reduced on the Balb/c and Bl/6 background. Elevated levels of IFN-γ (Th1 default development) in IL-4-deficient mice seems to be partially dependent for the

suppression of IL-5/eosinophilia observed in 129Sv mice, because doubly deficient mice (IL-4/IFN-γ R1) have an intermediate phenotype (eosinophilia is still reduced compared to wild-type but increased compared to IL-4ko mice). The number of eggs recovered from the feces of deficient mice infected with N. brasiliensis showed that an IL-4/type 2 response is beneficial, whereas an IFN-γ/type 1 response is unfavorable for the parasite. The requirement of eosinphils for anti-parasite responses and for the development of airway-hyperreactivity will be discussed.

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Session III: Cytokine dependent effector functions

Chairmen: Mariano Esteban and F.Y. Liew

The Role of Cytokines and Nitric Oxide in Infections

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There is considerable current interest in the role of cytokine inducible nitric oxide synthase (iNOS) in a variety of biological functions. Using a strain of iNOS-deficient mice, we have investigated the role of NO in leishmaniasis, malaria, *Staphylococcus aureus* and herpes simplex (HSV)-1 infections. iNOS-deficient mice are highly susceptible to these infections compared to wild type or heterozygous mice. Interestingly, in all cases, the mutant mice developed significantly higher Th1-type of responses compared to the resistant heterozygous mice, producing higher concentrations of IFN-γ and lower concentrations of IL-4. This suggests that NO could have a negative feedback effect on Th1 cell development.

Peritoneal macrophages from the infected iNOS-deficient mice produced significantly higher concentrations of IL-12 compared to those of the heterozygous mice. Furthermore, Human (THP1) and murine (J774) macrophage cell lines expressed markedly enhanced amounts of IL-12 message when incubated with an inhibitor of NOS (L-NMMA). This can be completely abolished by the presence of an NO donor (SNAP). These results therefore demonstrate that NO inhibits the transcription of IL-12 which is required to drive the development of Th1 cells. This may be an important regulatory mechanism preventing the over-expansion of Th1 cells which are implicated in a range of immunopathologies.

Pathogens have evolved complex strategies to ensure survival in an immunologically hostile host environment. In the case of *Leishmania*, the parasite can modulate the synthesis of NO by infected macrophages using glycoinositol-phospholipids (GIPLs) and lipophosphoglycan (LPG), two of the major groups of surface glycolipids of the parasite. Furthermore, purified LPG can inhibit the expression of IL-12. These strategies contribute significantly to the survival of the parasites.

ROLE OF INTERFERON-INDUCED ENZYMES IN THE CONTROL OF VIRAL INFECTIONS.

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Interferons (IFN), exert a wide range of biological effects in cells leading to antiviral, antitumor and immunomodulatory actions (1). About 20 IFN-inducible genes are activated in response of the cells to IFN, but the role of these gene products in the biological function of IFN is now beginning to be elucidated for some of them. Of the IFN-inducible genes, the two double-stranded RNA-dependent enzymes, protein kinase (PKR), 2-5A synthetase/RNase L system and nitric oxide synthase (iNOS), are thought to play an important biological role in the antiviral and anticellular functions of IFN, but direct evidence is lacking. To show the antiviral function of these IFN-inducible genes, we have generated vaccinia virus (VV) recombinants expressing individually each gene. Control of gene expression is regulated by either the E.coli lac I repressor/operator system or by the bacteriophage T7 RNA polymerase. Infection of cells with VV recombinants results in gene expression, if IPTG is added to cell cultures or if T7 RNA polymerase is produced. When PKR is induced, there is a dramatic decrease in virus replication (2), as well as induction of apoptosis (3). We provide evidence that inhibition of virus replication is due to phosphorylation of the alpha subunit of the eukaryotic initiation factor eIF-2, while activation of apoptosis requires a different pathway, a cellular protease that cleaves the death protein polyA-ribose polymerase (PARP). These effects are not observed with the catalytically inactive point mutant form of PKR (lys--arg 296) (4). When 2-5A synthetase is induced, there is limited inhibition of virus replication. However, expression of RNase L alone leads to significant inhibition of virus replication and this effect is enhanced by co-expression of 2-5A synthetase and RNase L. Inhibition of virus replication is due to rRNA breakdown. When iNOS is induced, there is a marked inhibition of virus replication at the level of DNA synthesis (5), as well as activation of apoptosis.

Our findings show that PKR, 2-5 A synthetase/RNase L and iNOS participate in the antiviral and anticellular effects of IFN. The extent of the various biological effects appear to be regulated by specific inhibitors. By modulating these various effects we might be able to enhance the therapeutic efficacy of IFN in infectious diseases.

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MIMICKING AND SYNERGISM OF CECROPIN A-MELITTIN HYBRID PEPTIDES WITH MACROPHAGE ACTIVATING CYTOKINES. NOPRODUCTION AND LEISHMANICIDAL ACTIVITY

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The cecropin A-melittin hybrid peptide CA(1-8)M(1-18) shows a strong lethal activity on Leishmania sp. promastigotes at micromolar concentrations by a membrane permeabilization mechanism; by contrast, amastigotes in vitro are much less susceptible to direct permeabilizing action of the peptide, but they were killed when infected macrophages are fed with liposome - encapsulated peptide. This suggest an indirect effect, and in fact the peptide is able to induce transcription of iNOS as well as production of NO in treated Raw cells at peptide concentrations lower than 5 μM; higher concentrations induce macrophage necrosis as measured by LDH activity in culture supernatant. At concentrations around 1 μM the peptide also induces apoptosis in the macrophage. CA(1-8)M(1-18) interacts with LPS, and antagonizes its NO induction, even when LPS addition is previous the peptide, likely because the induction of other signalling pathways; although they are under progress, the peptide induces a sustained increases in intracellular Ca^{2-}, which could play a role in this phenomenon. On the other hand , the peptide sinergizes with interferon-γ and TNF-α in NO production. Induction of NO levels by other shorter hybrid peptide analogues are lower and proportional to the their leishmanicidal activity.

The role of the peptides as macrophage activating agents to bypass situations where the supply of activating cytokines is decreased is discussed.

Inhibition of IFN-α/β activity by a soluble and membrane-bound IFN-α/β receptor encoded by vaccinia virus

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Poxviruses encode a broad range of proteins that interfere with host immune functions. Virulence factors that block complement activation, the antiviral effects of interferons (IFNs) or the activity of cytokines have been found, including soluble versions of receptors for tumour necrosis factor, interleukin-1β, IFN-γ and IFN-α/β. These virus-encoded cytokine receptors have a profound effect on virus pathogenesis and enable the study of the role of cytokines in virus infections.

The vaccinia WR B18R protein is secreted from infected cells and functions as a soluble receptor for IFN-α/β. In contrast to the cellular counterparts, which have fribronectin type III domains and are highly species specific, B18R has three immunoglobulin domains and binds IFN-α/β from several species.

We have found that after secretion B18R binds to both uninfected and infected cells. The B18R protein present in the cell surface maintains the binding properties of the soluble receptor, binding IFN-α/β with high affinity. B18R-coated cells are protected from the anti-viral state induced by IFN-α/β, presumably because B18R competes with cellular receptors for binding IFN-α/β. The replication of a B18R deletion mutant in tissue culture is restricted in the presence of IFN-α whereas the wild type virus replicates normally.

This represents a novel strategy of virus immune evasion in which secreted IFN-α/β receptors not only bind the soluble cytokine but also protect the uninfected cells from the anti-viral effects of locally produced IFN-α/β, maintaining the cell susceptibility to virus infections. This will help vaccinia virus to replicate and spread in the host.

Session IV: Role of cytokines in regulation of parasitic infection

Chairmen: Manuel Fresno and Fred D. Finkelman

HOST-PARASITE INTERACTIONS IN *TRYPANOSOMA CRUZI* INFECTIONS. ROLE OF CYTOKINES

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Intracellular replication of the protozoan parasite *Trypanosoma cruzi* inside macrophages is essential for the production of the disease and the development of the parasite. The parasite is able to replicate in the cytoplasm of primary resident macrophages but it is killed by activated macrophages. Th1 or Th2 T cell lines, could be established from T.cruzi-infected Balb/c mice which specifically proliferated to parasite antigens. Culturing Balb/c macrophages with Th1 clones or their cell-free supernatant but not with Th2 cell lines activated their trypanocidal activity. Furthermore, the trypanocidal inducing ability of Th1 supernatants was completely abrogated by neutralizing anti-IFN-γ and partially abrogated by neutralizing anti TNF-α antibodies. Furthermore, TNF-α had a synergistic effect with IFN-γ on the activation of *T. cruzi* killing and on the production of nitric oxide (NO) by macrophages whereas TNF-α was less effective although it was also synergistic with IFN-γ. More interestingly, both the NO production and the trypanocidal activity but not the superoxide generation induced in macrophages by TNF-α or IFN-γ alone or in combination, were inhibited by N-monomethyl-L-arginine (N-MMLA), a competitive inhibitor of NO synthase activity. Furthermore, a good correlation between the levels of NO production but not with superoxide generation and trypanocidal activity induced by different lymphokine preparations was found. I agreement with this, infected Balb/c mice which were able to control *T.cruzi* infections with a poorly virulent strain, G, showed greatly enhanced parasitemia and mortality when treated with N-MMLA.

In addition, differences in cytokine secretion by spleen macrophages were observed during "in vivo" infection of Balb/c mice with different strains. Spleen cells from the mice infected with the highly virulent strain MC were able to produce less TNF-α that those infected with the G strain.

Uninfected macrophages were able to secrete large amounts of TNF and IL-1 upon stimulation with LPS. However, infected macrophages have an selectively impaired ability to secrete TNF but not IL-1. This effect takes place at the level of translation since mRNA levels of TNF were greatly reduced whereas IL-1β mRNA levels remain basically unaffected.

Moreover, extracts of *T. cruzi* membranes were also able to mimic this effect when added to macrophages. Analysis pointed out that a recently described GPI-anchored "mucin-like", AgC10, as the protein responsible of this activity. This protein interacts with L-selectin on the membrane of macrophages and seems to be required for the entry of *T. cruzi* on those cells. This AgC10-L-selectin interaction alters signal transduction events in macrophages that not only affects their ability to secrete lymphokines but also the ability to costimulate T cell cultures, since Agc10 also induces a potent immunosuppression of anti-CD3 mediated T cell proliferation and IFN-γ secretion.

Altogether those data suggest that activation of macrophages by IFN-γ and TNF-α produced by Th1 cells is important to control *T. cruzi* infection. However, *T. cruzi* has evolved mechanisms to evade those response by altering the activating and cytokine secretion ability and the costimulatory activity of macrophages

DETECTION OF CYTOKINE PRODUCING CELLS IN HEARTS FROM MICE INFECTED WITH *TRYPANOSOMA CRUZI*.

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We demonstrate in this study the advantages of using an intracellular *in situ* technique for immunocytochemical staining of inflammatory infiltrates in hearts of *Trypanosoma cruzi* infected CBA mice. This mouse strain develops a defined chronic heart disease upon infection with *T. cruzi*.
We are using a methodology that measures cytokine production, enables analysis on single cell level and distinction between producer and responder cells. We analysed the major pathological target organ of Chagas disease, the heart, and measured the synchronous productions of various cytokines at set time points during the course of infection. We also stained and characterized the CD markers of the inflammatory infiltrates, obtaining quantitative data by using computerized image analysis. Cellular infiltrates were recorded in hearts from both acute and chronic stages, but were not observed in control hearts. In the acute heart, CD8 cells predominated, with associated production of IL-4, IL-6 and TNF-α. In the chronic heart cytokine production was characterized by IL-4, IL-5, IL-6 and TNF-α and numbers of CD4 and CD8 cells were more equivalent. At this stage, calcified infarctions and associated fibrosis were apparent, mimicking chronic human Chagas heart pathology. We consider the CBA mouse an appropriate model of chronic *T. cruzi* infection, and suggest that local cytokine production measured by *in situ* immunocytochemistry methodology reflects establishment of heart pathology.

Immune responses in an experimental malaria infection.

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Mice lacking components of their immune system or cytokines because of targeted gene disruption are powerful tools with which to investigate immune responses in infectious disease models. In an experimental malaria infection, *Plasmodium chabaudi chabaudi* in mice deleted of different subpopulations of T cells have provided convincing evidence that the critical cells involved in protective immunity to the erythrocytic stages of infection are αβ CD4+ T cells.

Earlier studies suggested that CD4+T cells were sufficient for the control of erythrocyte parasites without a requirement for B cells and antibody. More recently studies in definitive models of B cell deficiency where B cells are lacking because of disruption of either IgM(μ-MT or the junctional region of immunoglobulin heavy chain (JHD) clearly show that early control of the acute parasitemia is possible without B cells. However, elimination, control of chronic infection and resistance to a secondary challenge infection are absolutely dependent on B cells. Studies in μ-MT mice have also demonstrated that B cells may be important in regulation of T cell responses. In the chronic phase of infection seen in these mice there is a substantial increase in the number and proportion of γδ T cells in spleen and peripheral blood. Additionally there is a sustained CD4+Th1 response characterised by increased levels of IFN-γ. The increase in γδ T cells appears to be the result of chronic parasitemia since reduction of parasitemia by treatment with chloroquine restores the normal levels of these cells. By contrast the Th1 response may be a direct result of the lack of B cells, as reconstitution with B cells but not reduction of parasitemia with chloroquine, results in switch to Th2 responses .

Similar studies with cytokine-defective mice show that in the absence of IL-4 and IL-6, the receptors for IFN-γ and TNF-α that recovery from a primary infection is possible, suggesting either that immunity is independent of these cytokines or that through redundancy in the immune system, compensatory mechanisms have been upregulated. Regulatory cytokines such as IL-10 and IL-12 play key roles in the differentiation of the subsets of CD4+T cells. Female IL-10 knock-out mice infected with malaria are very susceptible to infection, with a significantly increased anemia and a 60 to 70% mortality rate. Death normally occurs within 20 day of infection and resembles lethal endotoxin shock. Analysis of cytokines in these mice showed that IFN-γ production was significantly up-regulated compared with wild-type mice, suggesting that inflammatory pathways normally down regulated by IL-10 may be responsible for the lethal outcome of a *P. chabaudi* infection. These studies maybe important in the immunopathology associated with malaria infections.

PROTECTIVE EFFECT OF IL-4 AGAINST GASTROINTESTINAL NEMATODES

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BALB/c mice infected with the GI nematode *Heligmosomoides polygyrus* develop a chronic 1° infection, but limit 2° infections with the same parasite. Protection against a 2° infection is blocked by a neutralizing anti-IL-4 receptor mAb, while mice can be cured of a primary infection by treatment with IL-4. IL-4-mediated host protection acts on the adult parasite, is primarily antibody- and B cell-independent and is only partially dependent upon the presence of a specific immune system, since decreases in worm burden and egg production are seen in IL-4-treated SCID mice.

IL-4 is not required for BALB/c mice to expel *N. brasiliensis*, but can induce *N. brasiliensis* expulsion by SCID mice, or anti-CD4 mAb-treated BALB/c mice, which otherwise fail to expel this parasite. IL-4 must mediate expulsion by actions on the host, rather than directly on the worm, because the effects of IL-4 on worm expulsion are blocked by a mAb to the mouse IL-4 receptor and because IL-4 does not induce worm expulsion from mice that lack a functional gene for Stat6, a molecule that is important in IL-4 signal transduction. Observations that IL-4 is only partially effective at inducing anti-CD4 mAb-treated, mast cell-deficient mice (w/w^v mice or mice treated with anti-c-kit mAb), or 5-lipoxygenase-deficient (5LO KO) mice to expel *N. brasilensis* suggest mast cell and leukotriene involvement in IL-4-induced worm expulsion. Indeed, mast cell products, induced by IL-4, change gut physiology in a way that may inhibit worm survival. Increased non-propulsive intestinal contractions are seen in IL-4-treated BALB/c mice and mice given a challenge infection with *H. polygyrus* , but not in IL-4-treated w/w^v mice, 5LO KO mice or *H. polygyrus*-infected, anti-IL-4 receptor mAb-treated mice, and can be blocked in vitro by a specific inhibitor of the leukotriene D4 receptor.

In addition to induction of increased smooth muscle contractility, IL-4 treatment and *H. polygyrus* infection are associated with an increased intestinal chloride secretory response to the mast cell-produced secretagogue PGE2, which can be blocked in *H. polygyrus*-infected mice with anti-IL-4R mAb, with increased intestinal fluid content, with decreased resistance to intestinal ion flow, and with decreased sodium absorption in response to glucose.

In sum, our observations indicate that IL-4 can protect hosts against gastrointestinal nematode infections and suggest that some of the protective effects of IL-4 are T and B cell-independent and are mediated by mast cell products that result in changes in gut physiology (increased intestinal contractions and net fluid content) that may interfere with parasite nutrition.

NEW ASPECTS OF Th CELL POLARIZATION. G. Thyphronitis E. Comoy, A. Capron and. M. Capron. U167 INSERM, Institut Pasteur de Lille, France.

In the last few years, many studies have shown the importance of polarized immune responses (i.r.) on the outcome of several parasitic, infectious and allergic diseases. Based on today's knowledge, one may predict that Th cell polarization is a complicated process controlled by a number of factors including: the nature of the antigen and of the APCs, accessory molecules expressed on APCs that deliver different co-activation signals to T cells, cytokines produced -by lymphocytes or other cell types- early after exposure to a pathogenic agent or immunization with an antigen, etc. The sum of these different signals, will determine which cytokine genes will be activated and expressed by Th cells. Two lines of research are developed in our laboratory to better understand Th polarization.

First, we examined whether eosinophils have immunoregulatory potential. It is indeed well known that eosinophils are effector cells involved in parasitic infections, allergic manifestations and in most inflammatory processes. However the possibility that they exert immunoregulatory functions has not been thoroughly examined. By immunostaining, and mRNA analysis we demonstrate that the immunoregulatory cytokines, IFNγ, IL-10, and IL-4 are produced by eosinophils. The intracellular content of cytokines was dramatically higher (up to 1000-fold) in eosinophils, compared to PBMC, suggesting a storage process by eosinophils. Thus, our results indicate that through cytokine release eosinophils might exert wide range of functions, including T cell polarization.

In a different line of research, we established a model system in which polarized immune responses were obtained against protein antigens. Mice were immunized with the Schistosoma mansoni Glutathion-S-transferase (Sm28-GST, a vaccine candidate) using different immunization protocols. These experiments have shown that different sets of cytokines were expressed depending on the delivery system employed. Thus, immunization with Sm28-GST in alum induced a typical Th2 profile with high IL4 production and specific anti-Sm28-GST antibodies of the IgG1 isotype. In contrast, when genetically modified, Salmonella typhimurium expressing the Sm28-GST was used for immunization, a Th1 type of response was observed with high IFN-γ and specific IgG2a responses. A mixed cytokine profile (IFN-γ + IL4) was observed when Complete Freud's Adjuvant was used for immunizations. To determine whether the observed cytokine profiles were "dictated" by the delivery system, we examined the type of the response generated against Tetanus toxoid fragment C (TTC) after immunizations using the same adjuvants and vector system (Salmonella also express TTC). Indeed, the cytokine and Ig isotype patterns expressed against TTC were very similar to the one's observed against Sm28-GST. These results strongly suggest that the type of immune response against some protein antigens is not dependent on innate antigenic properties but rather determined by "environmental" factors.

The experimental model described, is a useful one for studying mechanisms involved in the polarization of the immune response against protein antigens. We are presently examining two important questions: 1) whether the profile of

cytokines produced after primary immunization, will persist in subsequent exposures to the same antigen, and 2) by which mechanisms allergens polarize immune response to a Th2 profile. Preliminary data show that initial polarization persists even when rappel immunization is done using the opposite polarizing adjuvant. Regarding allergen induced polarization, our results suggest that allergens polarize immune responses mainly by inhibiting early Th1 (IFN-γ) cytokine production.

SCHISTOSOMA MANSONI EGG-INDUCED EARLY IL-4 PRODUCTION BY PERITONEAL EXUDATE CELLS IS DEPENDENT ON IL-5 AND EOSINOPHILS.

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The differentiation of antigen-specific Th2 cells is known to be largely dependent on the presence of IL-4 at the time of initial Th cell activation. The eggs of the trematode parasite *Schistosoma mansoni* provoke vigorous antigen-specific host Th2 responses. It is hypothesized then, that the initial host response to schistosome eggs would include the early production of IL-4. We have previously shown that peritoneal exudate cells (PEC) collected from naive mice 12 hours following intraperitoneal (i.p.) injection with schistosome eggs both transcribe the IL-4 gene and secrete IL-4 at higher levels than do PEC from PBS injected mice. Moreover, the secreted IL-4 is rapidly bound by either membrane or soluble IL-4 receptor (IL-4R), as evidenced by the inability to measure PEC production of IL-4 in the absence of anti-IL-4R mAb. *S. mansoni* egg injection also results in the rapid influx of eosinophils into the peritoneal cavity. The timing of the peritoneal eosinophilia corresponds with the peak of early IL-4 production by PEC. The egg-induced elevation in IL-4 and peritoneal eosinophilia was evident in nude, $CD4^+$ cell-depleted and β2-microglobulin knockout animals, but not in mast-cell deficient (W/W^v) or IL-5 knockout mice. To address whether or not IL-4 was necessary for the early eosinophil infiltrate at the site of antigen deposition, differential counts on PEC from egg-injected wild-type and IL-4 knockout mice were performed and no difference in peritoneal eosinophil numbers was observed. Furthermore, peritoneal and serum levels of IL-5 in both wild-type and IL-4 knockout animals were significantly increased over that of PBS injected animals. Taken together, these data strongly suggest that egg-induced IL-5 but not IL-4 plays an essential role in recruiting eosinophils to the site of antigen deposition. In addition, eosinophils either make or indirectly promote egg-induced early IL-4 production by PEC. Work is currently underway to investigate the relative importance of this IL-5-dependent IL-4 for the developing egg antigen-specific immune response.

Session V: Role of cytokines in initiation and regulation of cellular immunity

Chairmen: Giorgio Trinchieri and Alan Sher

INTERLEUKIN-12: A PROINFLAMMATORY CYTOKINE WITH IMMUNOREGULATORY ACTIVITIES by Giorgio Trinchieri, The Wistar Institute, Philadelphia, PA 19104

Interleukin-12 (IL-12) is an heterodimeric cytokine produced by phagocytic cells and other antigen-presenting cells in response to infections and other antigenic stimulations. The major target cells of IL-12 are natural killer cells and T lymphocytes, on which IL-12 induces production of cytokines, particularly interferon-γ (IFN-γ), proliferation, and enhancement of cytotoxic activity. IL-12 is rapidly produced when phagocytic cells or antigen-presenting cells are activated by infection, bacterial products or exposure to antigen-activated T cells and induces production of IFN-γ, which acts as a potent activator of the phagocytic cells. This early proinflammatory effect of IL-12/IFN-γ sets the stage early in an immune response for the ensuing antigen-specific T cell response. Thus, IL-12 serves as a bridge between the innate resistance and adaptive immunity. Overall, IL-12 and IFN-γ are required for efficient generation of Th1 cells, which produce IFN-γ and IL-2 and favor cell-mediated immunity and phagocytic cell activation, in contrast with IL-4, which is the cytokine required for generation of Th2 cells, which produce IL-4 and IL-10 and favor humoral immunity. However, IL-12 has a priming effect on T cells for high production not only of the Th1-type cytokine IFN-γ, but also of the Th2-type cytokine IL-10. The presence of IL-12 during the first several days of *in vitro* clonal expansion in limiting dilution cultures of polyclonally stimulated human peripheral blood "naive" CD45RO$^-$ T cells also induces stable priming for high IL-10 production in CD4$^+$ and CD8$^+$ clones. Priming for IL-4 production, which requires IL-4, was maximum in cultures containing both IL-12 and IL-4. IL-4 modestly inhibited the IL-12-induced priming for IFN-γ, but almost completely suppressed the priming for IL-10 production. A proportion of the clones generated from "memory" CD45RO$^+$ cells produced some combination of IFN-γ, IL-10, and IL-4 even in the absence of IL-12 and IL-4, suggesting *in vivo* cytokine priming; virtually all CD4$^+$ clones generated from either CD45RO(-) or (+) cells, however, produced high levels of both IFN-γ and IL-10 when IL-12 was present during expansion. The ability of IL-12 to prime CD4$^+$ and CD8$^+$ clones for IFN-γ and IL-10 production was also observed in PBL from 10 HIV$^+$ patients at various stages of the disease. These data suggest that intermediate types of Th cells, probably with regulatory functions in immune response and autoimmunity, may be generated in the presence of IL-12.

Induction of IL-12 by *Toxoplasma gondii* requires two biochemically distinct parasite signals.

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Toxoplasma gondii is a protozoan parasite which early in infection induces a strong IFN-γ dependent cell-mediated response that limits the intracellular replication of tachyzoites thereby insuring host survival. Interleukin-12 plays a key role in this innate resistance mechanism by stimulating NK and $CD4^+$ T cells to produce IFN-γ. Macrophages are thought to be the major source of IL-12 in the response and produce the monokine when infected with live tachyzoites or exposed to a soluble parasite extract (STAg). We have been characterizing the tachyzoite molecules responsible for IL-12 induction and comparing them with those triggering the production of TNF-α, another monokine involved in host resistance. These activities are present in STAg as well as tachyzoite membrane fractions and are both heat stable (5, 100°C) but highly sensitive to periodate oxidation. In contrast, the IL-12 and TNF-α inducing factors differ markedly in their susceptibility to protease digestion, the former being enzyme sensitive and the latter resistant. While normally insoluble in organic solvent, the TNF and residual IL-12 inducing activities are readily extracted into butanol after proteolysis and have been partially purified by octyl-sepharose and thin layer chromatography. The resulting fractions possess high levels of TNF-α but only minimal IL-12 inducing capacity. Nevertheless, upon addition of IFN-γ to the macrophages, these same glycolipid fractions induce high levels of IL-12. The above observations suggest that the protease sensitive element required for IL-12 stimulation functions by inducing IFN-γ or providing an equivalent macrophage priming signal. Since macrophages from class II MHC knock-out mice are also deficient in their IL-12 but not TNF-α response to *T. gondii*, the relevant protein would appear to require interaction with this cell surface molecule. We currently believe that a superantigen activity previously identified by us in tachyzoites is likely to be the class II dependent protease sensitive element necessary for IL-12 induction. This factor appears to be biochemically distinct from the parasite glycolipid molecules which serve as the trigger for monokine induction.

IL-12 production as a first line mechanism of defense in infectious diseases

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Interleukin-12 (IL-12) is a heterodimeric cytokine of 70 kDa (p70) composed of two unrelated chains, p40 and p35, encoded by separates genes. The production of IL-12 during the early inflammatory response to pathogens participates in setting the stage and profoundly influences the characteristics of the ensuing adaptive immune response against pathogens. The activity of IL-12 induces generation of Th1 cells, which produce IFN-γ and IL-2 and favor cell mediating immunity, macrophage activation and production of the opsonizing IgG2 isotype, and inhibits the generation of Th2 cells, which produce IL-4, IL-5 and IL-10 and favor humoral immunity and production of IgG1, IgE, and IgA isotypes. The presence of exogenous or endogenous IL-12 has been shown to elicit protective immunity in several vaccination protocols. Here, we analyzed to what extent endogenous IL-12 production could be modulated by the genetic background, by the adjuvant used, and by the cell type targeted.

In mice, genetic differences in IL-12 production can influence the development of resistance in response to vaccination. Indeed, mice from the P strain fail to develop significant resistance to *Schistosoma mansoni* infection after vaccination, and this failure correlates with defects in macrophage larvicidal activity. Splenocytes from either naive or vaccinated P mice secrete less IL-12 than splenocytes from vaccine-responsive strains such as C57BL/6. The impaired production of IL-12 by P mice splenocytes correlates with an increased synthesis of IL-4 and IL-10, two cytokines which down regulate macrophage activation. Nevertheless, splenocytes from both mouse strains were equally responsive to IL-12, as measured by IFN-γ synthesis.

IL-12 synthesis was also found to mediate the adjuvant effect of trehalose dimycolate (TDM), a cell wall glycolipid of Mycobacteria. Although present in minor quantities, TDM is a potent immunomodulator which limits tumor growth and elicits protection against several microorganisms. We demonstrated that TDM injected intraperitoneally induces a rapid synthesis of IL-12p40 and IFN-γ mRNA by peritoneal cells. Moreover, inhibition of IL-12 or IFN-γ activity by neutralizing mAbs blocked antiproliferative activity and the nitric oxide release by TDM-elicited macrophages. Thus IL-12 and IFN-γ production represent an obligatory step in TDM-induced activation of mouse peritoneal macrophages.

Finally we demonstrated that not only macrophages but also epithelial cells, especially enterocytes, were able to produce IL-12. Indeed, the murine enterocytic cell line MODE-K produces IL-12 upon cytokine (IFN-γ), parasite (*Toxoplasma gondii*) and bacterial (*Salmonella typhimurium*) stimulation. These results suggest that IL-12 production by epithelial cells could influence the onset of the Th1/Th2 balance during microbial infection.

Neutrophil-Producing IL-12 as Initiator of Th1 Cell-Mediated Immunity in Murine Candidiasis

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Recent studies in mice have shown that the outcome of mucosal and systemic *Candida albicans* infection is largely dependent upon which $CD4^+$ T helper (Th) cell subset predominates (1-3). Protective Th1 responses to virulent *C. albicans* cells are observed in genetically resistant mice after vaccination with a live vaccine strain of the yeast, which will instead induce a Th2 response in genetically or otherwise susceptible mice. Th1 differentiation in vivo requires the concerted actions of at least four different cytokines, IFN-γ, TGF-β, IL-6, and IL-12 (4-6). Although neutralizing antibodies to these cytokines may block Th1 development, neither IFN-γ, TGF-β or IL-6 are individually predictive of, or selectively associated with, Th1 development, as is the case for IL-12. IL-12p40 specific transcripts were found to be persistently expressed only in healer mice, being negatively regulated by the Th2 cytokines. These findings may predict a decisive role of IL-12 in initiation of Th1 development in candidiasis. Analogous to the effect of IL-12 neutralization, depletion of neutrophils in resistant mice leads to the onset of Th2 rather than Th1 responses, suggesting that the latter cells participate in *Candida*-driven Th1 development (7). As it is becoming clear that neutrophils may be considered not only as terminally differentiated effectors, but also as capable of synthesizing different cytokines (8), in the present study we investigated the immunomodulatory role of neutrophils in the generation of Th-dependent immunity in mice with candidiasis. Because the induction of protective Th1 cell responses in healer mice requires the activity of IL-12 in the relative absence of the counter-regulatory cytokines IL-4 or IL-10, the neutrophil's ability to release these cytokines was evaluated both in vitro and in vivo in mice with disseminated candidiasis. It was found that neutrophils were endowed with the ability to secrete both IL-10 and IL-12, as assessed by cytokine gene expression, protein production in vitro and intracellular cytokine localization. The neutrophil's secretion of these cytokines was differentially regulated in mice with healing or nonhealing infection, being IL-12 predominantly produced in healer mice and IL-10 in nonhealer mice. Secretion of IL-10 was also increased as a result of exogenous IL-12 administration in vivo. Neutrophil depletion prevented the development of protective Th1 responses in healer mice, but this intervention could increase resistance of otherwise susceptible mice. Protective Th1 immunity could be efficiently restored in neutrophil-depleted mice by IL-12 administration, which resulted in cure of mice with disseminated candidiasis. These findings demonstrate that neutrophils, through release of cytokines, may play an active role in determining Th selection in mice with candidiasis, thus providing evidence for the interdependence between the innate and the adaptive specific immunity in *C. albicans* infection. In addition, their Th1 promoting role in vivo could be efficiently substituted by replacement therapy with IL-12.

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***Brucella* spp. release a specific, protease-sensitive, inhibitor of TNF-α expression active on human macrophage-like cells.** Emmanuelle Caron, Antoine Gross, Jean-Pierre Liautard, and Jacques Dornand. INSERM U-431, Université de Montpellier II, F-34095 Montpellier

Brucella spp. can establish themselves and cause disease in humans, but the mechanisms by which brucellae evade the antibacterial defenses of their host remain largely unknown. We previously reported that, unlike *Escherichia coli* K12, intracellular pathogens from the genus *Brucella* survive and multiply within U937-derived phagocytes. To determine whether *Brucella* infection modulated the production of TNFα, a central cytokine in the first-line of defenses of host against foreign organism, we measured the biological activity of this cytokine in the supernatant of U937 cell-derived macrophages infected with different *Brucella* strains. Unlike *E.coli*, none of the *Brucella* strains tested induced any measurable TNF-α release upon infection, whatever the bacteria have been preopsonized with anti-brucella IgG or not. RT-PCR analysis and cytokine measurements demonstrated that *Brucella*-infected U937 cells were activated to express IL-1β, IL-6, IL-8, TGFβ1 both at the mRNA and protein levels while they did not accumulate TNF-α mRNA. On the contrary, gentamycin (or chloramphenicol)-killed brucellae promoted a significant excretion of TNFα and all the other cytokines from U937-derived macrophages. In addition, we showed that exogeneously added TNF-α did restrict intracellular growth of virulent *Brucella*. When physically separated from macrophages, live brucellae impaired TNF-α production in *E. coli*-infected cells. Moreover, in agonist-activated macrophages, supernatants from *Brucella* cultures promoted an inhibition of the induction of both TNF-α expression and release, without affecting IL-1β, IL-6 or IL-8 induction. These phenomena, observed whatever the *Brucella* strains assayed, show that brucellae release some high m.w. factor(s) that specifically inhibits TNF-α expression in activated human macrophages. The proteic nature of the factor(s) was demonstrated by its heat- and protease-sensitiveness and HPLC chromatography, and could explain why U937-derived macrophages did release TNF-α when activated with gentamycin or chloramphenicol-treated brucellae. We also found that the *Brucella* factor(s) specifically acts on human macrophagic cells but not on murine macrophage-like cells. Our findings provide direct evidence that a secreted *Brucella* virulence factor(s) inhibiting TNF-α expression might contribute to the evasion of *Brucella* organisms from human antimicrobial defenses.

Session VI: Cytokine determinants of disease progression

Chairman: Enrico Maggi

MOLECULAR BASIS OF INFLAMMATORY RESPONSE. PHYSIOPATHOLOGICAL ROLE OF CYTOKINES

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We provided definite evidence that T cells similar to the murine Th1 and Th2 cells can be demonstrated in humans, as well. Th1, but not Th2, T cell clones (TCC) produce IL-2, IFN-γ and TNF-β, whereas Th2 cells synthesize IL-4 and IL-5. The most important concept is that different cytokine patterns lead to very distinct inflammatory responses. Th1 cells promote both macrophage activation that results in delayed-type hypersensitivity (DTH) and production of opsonizing antibodies, particularly required for clearance of infection caused by intracellular organisms (phagocyte-dependent response). Th2 cells provide optimal help for production of antibodies (IgE included) that adhere to mast cells and promote both mast cell and eosinophil activation (phagocyte-independent response). The signals modulating the in vitro development of TCC specific for bacterial and viral antigens are also examined. Preculturing T cells with bacterial antigen (inducing a Th1 response) plus IL-4, a clear cut Th0/Th2 shift of cytokine production by antigen-specific T cell lines and clones can be found. Similarily, the development of allergen-specific T cells into Th2-like TCC is markedly inhibited by IFN-γ plus anti-IL-4 antibody, IFN-α, TGF-β or IL-12. Polyinosinic/polycytidilic acid (Poly I: C), a double stranded RNA which can mimic several viral intermediates, exerts a powerful effect on the development of allergen-specific T cells into TCC with Th1-like cytokine profile directly inducing the production of IL-12 and IFN-α by monocytes. Since recombinant IL-1RA exerts the same effect of Poly I:C, we can hypotheze that IL-1 can synergize with IL-4 in the development of Th2 cells, whereas IFN-α and IL-12 can polarize the Th response towards Th1 cells. Also hormones present in the microenviroment can influence the development of Th1 or Th2 cells: progesterone in bulk culture in the presence of specific antigen endowes PPD-specific T cells to develop T cell lines and TCC able to produce IL-4 and IL-5, in addition to IFN-γ. On the other hand, relaxin modulates Th2 cells towards the production of Th1-type cytokines. An imbalance among different hormones can be crucial to maintain the local Th response (Th2-oriented) during pregnancy able to block foetal allograft rejection

Very recently we focuse our study on membrane and soluble markers of Th1 and Th2 responses. The lymphocyte activation gene (LAG)-3, a

member of the immunoglobulin superfamily, preferentially associates with TCC with Th1-type cytokine secretion, whereas the great majority of Th2 clones shows neither surface LAG-3 nor LAG-3 mRNA expression. Following activation, the majority of $CD4^+$ TCC also releases soluble LAG-3-related peptides and such a release correlates positively with the production of IFN-γ and inversely with the production of IL-4. LAG3+ IFN-g-producing CD4 cells were found in the lesional mucosa of patients with Crohn's disease, a condition characterized by a Th1-type response.

On the other hand CD30, a member of the tumor necrosis factor-receptor (TNF-R)/nerve growth factor (NGF) receptor superfamily, preferentially associates with CD4 (and CD8) TCC producing Th2-type cytokines. Th0 and Th2 clones show both CD30 mRNA and surface CD30 expression and release soluble CD30. We have also shown that CD30-CD30L interaction can exert an important role in the differentiation of Th2 cells. In the presence of anti-CD30L ab we can derive allergen-specific TCC with Th1-like profile, whereas the anti-CD30 Ab with agonistic activity shows an opposite effect on the development (towards Th2-type response) of PPD-specific TCC. Finally, CD30+ T cells may play a very important pathogenic role during the course of HIV infection. Indirect support for a bias towards Th2-type responses, recently proposed for HIV infection, was provided by the increased serum levels of sCD30 in early phases of infection, strictly related to a faster progression to full-blown disease. The elevated levels of sCD30 may be due to the presence of large numbers of CD8+CD30+ Th2-like TCC in the late phases of infection, or, more likely, to the continuous activation and/or death of CD4+CD30+ T cells. Support for this was provided by the demonstration that a proportion of in vitro HIV-infected CD4+ TCC (but not their noninfected counterparts) express CD30 antigen and, concomitantly with that, initiate to dye. We have also shown that CD30 triggering favors both the replication of HIV and the death of CD4+CD30+ cells. On CD4+ T-cell lines and clones generated from HIV-infected individuals the anti-CD30 agonistic mAb exhibited a strong synergistic effect on HIV replication induced by the anti-CD3 mAb. Furthermore, paraformaldehyde-fixed CD8+TCC consistently expressing CD30L, were able to increase HIV replication into CD4+ T-cell lines derived from HIV-infected individuals, and more importantly, this activity was abrogated by the addition of anti-CD30L mAb. These data strongly suggest that HIV infection promotes CD30 expression in CD4+ T cells and that triggering of CD30 by CD8+ T cells expressing CD30L, may influence both the HIV replication in infected CD4+cells and their death.

TYPE 1/TYPE 2 CYTOKINES IN HIV INFECTION: INFLUENCE ON APOPTOSIS AND DISEASE PROGRESSION.

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We have pursued the analysis of cytokine production profiles in HIV infected patients in the framework of the Type 1 to Type 2 shift recently hypotesized to be an important pathogenetic factor in the progression of HIV infection. We briefly summarize here our latest results

1) The TH1/TH2 hypotesis of HIV infection. New insights.

We suggested that the immune dysregulation observed in human immunodeficiency virus (HIV)-infected individuals during progression to AIDS could be accounted for by a shift from a type 1 to a type 2 cytokine profile (rev in 1, 2). Thus, we proposed that a dominant type 1 cytokine profile would be more protective against disease progression than a dominant type 2 cytokine profile. In additional studies regarding this hypothesis we have shown that IL-12 (a type 1 cytokine which is defective in HIV+ individuals) enhances in vitro antigen-stimulated proliferation, IL-2 and IFNg production of HIV+ patients (3). Additionally, in collaboration with the Clinica Endocrinologica, H.L. Sacco, Milano, we have proposed that the early endocrine alterations characteristic of HIV infection could be responsible for the type 1/type 2 shift. Thus, an increase in cortisol concentration would selectively suppress CMI and type 1 cytokines, whereas the concurrent decrease in the concentration of dehydroepiandrosteron would have the opposing effect of favoring the production of type-2 cytokines (4).

2) Programmed cell death and cytokines

To investigate how type 2 cytokines could account for a more rapid evolution of the disease we analyzed the influence of cytokines on programmed cell death (PCD). We verified the previous observation that HIV+ lymphocytes are particularly susceptible to TCR-induced PCD, and we observed that the extent of this PCD can be differentially modulated in vitro by type 1 and type 2 cytokines (5). Therefore, type 1 cytokines block T lymphocyte PCD whereas type 2 cytokines have either no effect or enhance in vitro T cell PCD (5). Additionally, PCD can be inhibited by antibodies against IL-4 and IL-10, and enhanced by anti-IL-12. Interestingly, TH1 but not TH2 lymphocytes are likely to be selective targets of PCD as only TH1 express the ligand for Fas (the interaction between Fas and its ligand induces PCD of mature lymphocytes). Thus, the increased susceptibility to PCD and the decline in CD4 counts characteristic of HIV infection could be related to a shift from the production of cytokines with a protective effect against PCD to the production of cytokines enhancing PCD. This would, in a self amplifying loop, worsen the imbalance of type 1 and type 2 cytokines by selectively killing type 1 cytokines-producing lymphocytes. Finally, we have shown that PCD in HIV+ individuals is induced by the interaction between CD4 and gp120 (and is prevented by pretreatment of PBMC with sCD4); is preferentially observed in CD4+ T lymphocytes; and is mediated by lymphotoxin (6).

3) Immunologic analyses in HIV+ individuals with different patterns of disease progression

We analyzed in vitro PHA-stimulated cytokine production by PBMC of adult HIV+ individuals with different patterns of disease progression (collaboration with the Clinica della Malattie Infettive H.L. Sacco, Milano). Thus, we analyzed cytokine production, prevalence of viral isolation, and surface markers expression of PBMC of 26 HIV+ long term non progressors (LTNP); 28 HIV+ patients with progressive HIV infection (PI); and 24 HIV- controls (HC). We observed that: 1) IL-2 and IFNg production is reduced in PI patients compared to HC and LTNP; 2) IL-4 and IL-10 production is increased in PI patients compared to HC and LTNP; 3) prevalence of HIV isolation is lower in LTNP compared to PI, and the primary viral isolates in LTNP show a slow/low (S/L) phenotype; and 4) the elevated production of type 2 cytokines is paralleled by an increase in CD57+ CD4+ CD7- lymphocytes. Thus, whereas a high IL-2, high IFNg/low IL-4, low IL-10 cytokine production pattern is present in HC and in LTNP HIV+, the progression of HIV disease is associated with low IL-2 low IFNg/high IL-4, high IL-10 cytokine profile; increased prevalence of HIV isolation; and augmented percentage of CD57+ CD4+ CD7- lymphocytes (7).

4) Immuno-virologic correlates of HIV infection.

Finally, HIV isolability, rate of viral replication, HIV phenotype, type 1 and type 2 cytokine production, and CD4 counts were cross-sectionally analyzed in 63 HIV-seropositive individuals to establish correlations between virologic and immunologic markers of protection and progression. We observed that these markers are tightly correlated. Thus, lack or low prevalence of HIV isolability and the presence of non-syncitium inducing strains are associated with the strongest type 1 cytokine production, the weakest type 2 cytokine production, and highest CD4 counts. Conversely, the isolation of highly replicating, syncitium-inducing HIV strains is associated with the weakest type 1 cytokine production, the strongest type 2 cytokine production, and lowest CD4 counts. Additionally, it was determined that the IL-2/IL-10 ratio best discriminates amongst different virologic scenarios. Therefore, the virologic and immunologic correlates of disease protection and progression are closely associated variables that clearly define two different subsets of HIV+ individuals (8).

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CLINICAL, IMMUNOLOGICAL AND VIROLOGICAL PARAMETERS IN CHILDREN WITH VERTICALLY ACQUIRED HUMAN IMMUNODEFICIENCY VIRUS (HIV-1) INFECTION SURVIVING LONGER THAN 8 YEARS.

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Children infected with HIV-1 do not necessarily develop AIDS and can be divided into long-term and short-term survivors. We examined long-term survival in children perinatally infected with HIV-1. We have studied 15 children who survived longer than 8 years [defined as long-term survivors (LTS)] and compared them with 6 children who died of HIV-1 related disease before 3 years [short-term survivors (STS)]. Group A: 9 LTS remained asymptomatic (Center for Disease Control [CDC] category N) with normal and stable $CD4^+$ T cell counts despite 8 to 12 years of HIV infection (35.44±3.09 % CD4). Group B: 6 LTS (CDC category A) with lower $CD4^+$ T cell counts (5.67±1.87 %CD4) who remain free of opportunistic complications within 3 years of the $CD4^+$ T cell count drop. This occurs even in absence of treatment. 6 STS had AIDS and low CD4+ percentages (11±3 $\%CD4^+$ T cells). HIV RNA in plasma was detectable in all of the 21 HIV infected children. The mean amount of HIV-1 in the plasma of the 9 LTS group A was lower than 6 LTS group B an lower than those found in the 6 STS children ($p<0.001$). Infectious HIV-1 could not be isolated from five children (from group A) despite multiple attempts using optimal protocols. Nevertheless, HIV-1 could be recovered from PBMC of the remained four children. The kinetics of replication in these four isolates was slow/low and none were considered to have a syncytium-inducing phenotype. By contrast, HIV-1 was isolated from PBMC all of 6 the children from group B. Two of those HIV isolates were slow/low and NSI. By contrast, four isolates were designated as rapid/high and were uniformly able to infected and induce syncytium formation (SI). All 6 STS children carried viruses with cytopathic biological phenotype (rapid/high replication rate and SI viral phenotype). Plasma samples from our LTS had broad viral neutralizing activity in general, especially when compared with the lack of neutralizing activity of plasma samples from STS children. Cytokine production of unstimulated and mitogen-stimulated PBMC were evaluated in the 15 LTS children. No differences in the production of IFN-γ and IL-2 (type-1 cytokines) and TNF-α were detected between group A and B. In contrast, IL-10 (type 2 cytokine) production was augmented in group B. In summary, in the group A LTS children, HIV-1 replication appeared to be well controlled, with low viral load in plasma. Repeated attempts to isolate infectious HIV-1 from five of the nine children were unsuccesful; in other four viral attenuation was evident. The levels of virus and the degrece of immunity observed in these children could serve as important guideposts for the therapeutic and imunoprophylactic efforts against AIDS. Four of six isolates from group B LTS children carried more cytopathic biological phenotype and were more likely to have low $CD4^+$ and mildly symptomatic infection. Moreover, this study documents that substantial numbers of HIV-1-infected children remained free of AIDS illnesses (even untreated) for long periods (LTS) . Whereas, at the initiation of the study, STS children with AIDS carried viruses with more cytopathic biological characteristics, had significantly higher viral load and lower $CD4^+$ cell counts. The associations observed here reinforce the important role played by host and viral factors in the pathogenesis of HIV-1 infection.

MECHANISMS IMPLICATED IN THE CELL GROWTH TRIGGERED BY IL-2 OR IL-4 AND INTERLEUKIN DEPRIVATION

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Clonal expansion of lymphocytes is initiated by the interaction of antigen with the specific clonally-distributed receptor, which triggers cytokine production and appropriate cytokine receptor expression. Subsequently, cytokine-cytokine receptor interactions allow lymphocytes to undergo proliferation.

TS1 is a murine T cell line which was originally isolated as an IL-9-dependent clone and later found also to respond to IL-4. Transfection of human IL-2 receptor α, β or $\alpha\beta$ chains allowed the generation of IL-2-responsive clones which stably express low, intermediate or high affinity forms of the IL-2 receptor. The availability of this cell line, which can be maintained in the presence of either IL-2 or IL-4, might be useful for scrutinizing the similarities and differences in IL-2 and IL-4-elicited signaling pathways and cellular responses. These two lymphokines are related to T helper subset differentiation patterns, namely the Th_2 population in the case of IL-4/IL-10 and the Th_1 subset for IL-2/IFN-γ. Thus, through its resemblance to an *in vitro* Th_1/Th_2 differentiation system, our cellular model opens the way to the analysis of the mechanisms and changes which control the physiological determination of immune responses.

Our results show the implication of the protein kinase C (PKC) ζ and ε isoforms in IL-2-induced signaling pathways. Interestingly, IL-4 promotes TS1$\alpha\beta$ cell proliferation in the presence of PKC inhibitors or antisense oligonucleotides specific for several PKC isoforms. Subsequent results suggested that, during IL-2 stimulation, ζPKC was redistributed from a subcellular cytosolic location to a cytoskeletal compartment corresponding to actin structures. This PKC isoform appeared to be a checkpoint in the actin cytoskeleton arrangement promoted by IL-2, but not by IL-4. In unravelling the signaling mechanisms connecting ζPKC activity to actin cytoskeleton organization in IL-2- but not IL-4-stimulated TS1$\alpha\beta$ cells, we identified that the Rho family of GTP-binding proteins, phosphatidylinositol 3 (PI3) kinase and ζPKC are critical components in the IL-2-induced proliferative pathway.

Finally, we have characterized Ras involvement in the TS1$\alpha\beta$ cellular responses to lymphokine stimulation and deprivation. First, the combined use

of a dominant negative Ras mutant and a tetracycline-controlled expression system has enabled us to define a critical role for Ras in the induction of proliferation and prevention of apoptosis by IL-2, but not IL-4. Second, we have found that Ras is activated upon lymphokine withdrawal, and that this is required for induction of apoptosis in lymphokine-deprived cells. Cell death in the absence of lymphokines can be prevented by bcl-2 expression, which is also switched on by IL-2. Thus, Ras activation leads to cell death unless bcl-2 is expressed. In concordance with results from others, we propose that IL-2 triggers three distinct signaling pathways, represented by Ras activation, bcl-2 expression and c-*myc* expression, respectively.

Conversely, IL-4 stimulates proliferation in the absence of Ras activation and bcl-2 expression, or in the presence of drugs that inhibit Rho or PKC. Signals transduced through the IL-4 receptor, which are still poorly characterized, are possibly mediated by tyrosine phosphorylation of the insulin receptor substrate-1-related molecule 4PS, which serves as a docking protein for adapter molecules. Interestingly, 4PS phosphorylation appears to be related to the absence of Ras activation in IL-4-stimulated cells. Although 4PS phosphorylation is certainly a proximal event to activation through the IL-4 receptor, the primary transducers, which would in fact be responsible for determining IL-2- or IL-4-related responses, are still unknown.

The results of these studies have allowed us to derive a model which accounts for the mechanisms implicated in proliferation or induction of programmed cell death, as well as the different sets of transducer molecules activated by IL-2 or IL-4. It is hoped that the comparative study of IL-2- or IL-4-triggered signals in a single cellular system will contribute to highlighting the key biochemical events which govern lymphocytic proliferative responses within a Th_1 or Th_2 framework.

Pathogenic role of TNFR2, but not of TNFR1, in experimental cerebral malaria

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The rapidly lethal syndrome of cerebral malaria (CM) develops in some strains of mice upon infection with *Plasmodium berghei* ANKA (PbA). A crucial mediator of the neurovascular lesions appears to be tumor necrosis factor (TNF), found in high amounts in both experimental (Grau *et al*, 1987) and human CM (Grau *et al*, 1989). The brain pathology of human CM is comparable to that observed in CM-susceptible mice, but the nature of the cells sequestering in the brain is different. Both human and murine syndromes are characterized by an upregulation of ICAM-1 on the vascular endothelium. This upregulation correlates with an increased trapping of parasitized erythrocytes in brain microvessels in human CM (Porta *et al*, 1993; Turner *et al*, 1994) and of leukocytes in experimental CM.

Our previous results have indicated that during CM, TNF is involved in the induction of ICAM-1 on brain MVEC, thereby leading to increased interactions between platelets, expressing the ligand of ICAM-1, namely LFA-1 (Grau *et al*, 1993). Subsequently, platelets will fuse to the endothelial cells, giving rise to a fragilization of the endothelium and to the expression of platelet markers as well as of LFA-1 on the fused cells, that can subsequently interact with ICAM-1 expressed on sequestering leukocytes (Lou *et al*, submitted).

Although TNF has been implicated in the pathogenesis of experimental cerebral malaria (CM), the respective role of its two types of receptors has not been established. A significant increase in the expression of TNF-receptor 2 (TNFR2, p75), but not of TNFR1 (p55), was found on brain microvessels at the time of CM in susceptible, but not resistant animals. Moreover, mice genetically deficient for TNFR2 (*Tnfr2°*) (Erickson *et al*, 1994) were significantly protected from experimental CM, in contrast to TNFR1-deficient (*Tfnr1°*) mice (Rothe *et al*, 1993), that were as susceptible as B6x129 control mice.

In order to identify the factors involved in the protection from CM conferred by the lack of TNFR2, we assessed in both knock-out and control mice 1) the serum concentrations of mediators that are critical for the development of CM, 2) the relative sensitivity of brain MVEC towards direct TNF cytotoxicity, and 3) the upregulation of ICAM-1 in the brain microvessels.

In mice from all three lines there was no significant difference in serum levels of TNF and IFN-γ. As expected, soluble TNFR1 (sTNFR1) and sTNFR2 were completely absent in the serum of the respective knock-out mice, whereas the serum level of the remaining sTNFR was normal.

Although the sensitivity towards the direct cytotoxic activity of TNF was significantly reduced in brain MVEC isolated from *Tnfr2°* mice, as compared to MVEC isolated from w.t.

mice, this was also the case for the *Tnfr1*$^{+}$ brain MVEC. Interestingly, the pronounced ICAM-1 upregulation and leukocyte sequestration, that typically occurs in the brain microvessels of CM-susceptible animals, was detected in brain microvessels of infected control and *Tnfr1*$^{+}$ mice -both of which developed CM- whereas no such ICAM-1 upregulation nor leukocyte sequestration were observed in *Tnfr2*$^{+}$ mice, which were protected from CM.

In contrast, the lungs of the B6x129, *Tnfr1*$^{+}$ and *Tnfr2*$^{+}$ mice isolated at the time of the CM syndrome, all showed increased ICAM-1 upregulation.

We thus conclude that TNFR2 signalled upregulation of ICAM-1 in the brain is of critical importance for the development of the neuro-immunological complications of experimental malaria (Lucas *et al*, submitted) and that, in contrast to most TNF-induced pathological reactions described so far, signalling by TNFR2 rather than by TNFR1 is critical for this syndrome.

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TNF in malaria: an evolutionary balance

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Early in the course of malarial infection, the host produces tumour necrosis factor and other cytokines. TNF is released by monocytes and macrophages in response to toxins that are released into the bloodstream when parasites rupture to release their progeny. The clinical result is a febrile illness which generally resolves within a few weeks, but excessive TNF production may lead to fatal complications such as cerebral malaria. This provides a paradigm for addressing some fundamental questions concerning the innate host response to infection. What parasite molecules stimulate TNF production? What causes the abnormally high levels of TNF production that lead to severe pathology? Is this genetically regulated?

A key question is whether individuals differ in their propensity to produce TNF in response to malarial infection. A rough measure of individual variation is provided by the amount of TNF produced by whole blood stimulated with whole parasite lysates. In a group of over 300 healthy European adults who were sampled on multiple occasions, we found stable differences of over ten-fold in TNF output. Preliminary results from a study of Gambian twins show that TNF responsivess is more concordant among monozygous than among dizygous twin pairs. These findings indicate that the TNF response to malaria has a genetic basis.

Approaching the same problem from a different direction, we have been investigating the relationship between polymorphism of the TNF promoter region and susceptibility to severe malaria in African children. In a large Gambian case-control, we found that homozygotes for the $-308_{g\rightarrow a}$ variant of the TNF promoter region had a substantially increased risk of developing cerebral malaria. More recently we have found another variant, $-238_{g\rightarrow a}$, to be associated with susceptibility to severe malarial anaemia in two populations of different genetic background. It is important to note that these associations are independent of variation in surrounding HLA class I and class II regions. We postulate that the two variant alleles act in different ways to modify the TNF response to malaria, and thus have different effects on cerebral malaria (an acute complication) and on severe malarial anaemia (which is more likely to be due to chronic or repeated infection). Cellular studies to test this hypothesis require the relevant stimulus, ie the major TNF-inducing toxin of *Plasmodium falciparum*. This we have purified by quantitative biochemical fractionation: it is a proteolipid structure, bioactive in the order of 100pM, which rapidly induces NF-κB binding to the TNF promoter region. The purified toxin is currently being utilised to analyse the effects of the -238 and -308 polymorphisms on reporter gene expression and DNA-protein interactions within the TNF promoter region.

Independent studies are yielding associations between TNF promoter polymorphisms and susceptibility to other infections, including leprosy and tuberculosis. For any given infection, there is likely to exist a level of TNF response that provides maximum protection with minimal risk of pathology. However the optimal host response will not be the same for all infections since, even within *P. falciparum*, parasite strains vary widely in their ability to induce TNF. High TNF producers may do well in one type of infection and poorly in another. If these considerations are correct, then it is likely that future studies will reveal a high degree of functional polymorphism in genes that regulate the innate host response to infection.

POSTERS

rHuGM-CSF EMPLOYMENT FOR OPPORTUNISTIC DRUG-RESISTANT INFECTIONS IN AIDS

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Objective: To determine the efficacy and safety of rHuGM-CSF in HIV positive patients with opportunistic drug-resistant infections .

Methods: Since May 1995 all patients with oral or/and oesophageal candidiasis resistant to treatment with systemic imidazoles and patients affected by cryptosporidiosis unresponsive to paromomycin 4,3g/day observed in our Departments have been enrolled in an open, observational pilot study. Main inclusion criteria were seropositivity for HIV, leucopenia, antiretroviral treatment. Main exclusion criteria were creatinine > 2 mg/dL, pregnancy, presence of *Candida kruzei* strains, presence of causes of diarrhoea other than cryptosporidiosis, myeloproliferative diseases, treatment with drugs that could increase the growth of candida (i.e. corticosteroids) or could decrease the efficacy of azole therapy (i.e. rifampin, rifabutin, H2 antagonists). Drug resistance was assessed on a clinical basis: the patients had assumed all the commercial azoles at the highest dose for at least two weeks each without clinical benefit (candidiasis) or had been treated with paromomycin 4,3 g/day for at least 10 days without minimal reduction of the diarrhoea (cryptosporidiosis). All the patients continued the antimicotic or antiparasitic drug they were assuming at the time of enrollment. Complete medical history, physical exam, oral/ pharingeal culture exam (candidiasis), direct research of parasytes on stool (cryptosporidiosis) and blood work (including HIV antigenemia) were performed at time 0, 7, 14, 21, 28 and 42 of the study. Biliary fluid for cryptosporidium was collected at time 0 and 28.

Results: *Candidiasis:* Eight patients have been enrolled until now; 6 patients receiving zidovudine and 2 zalcitabine, presenting less than 50 CD4+ cells/mmc, treated for 1 to 3 years with every kind of antifungal treatment actually in use except i.v. amphoterycin B (ketoconazole, itraconazole, oral or i.v. fluconazolo), although differing for the duration of the single therapies and the order of the sequence. All the patients were assuming the last drug (in three cases itraconazole and in the others fluconazole) from at least 14 days without improvement. GM-CSF was administered 150 µg per day (weight < 60 Kg) or 300 µg per day (weight > 60 Kg) for 10 days. Six patients showed complete clinical and culture resolution in 10 days, patient 7 had only partial clinical regression while patient 8 had no clinical improvement over the treatment time.

Cryptosporidiosis: Two patients have been enrolled until now, both receiving zidovudine (17 and 21 months) and both with less than 50 CD4+ /mmc. One had been treated with paromomycin 4,3g/day for ten days, while the other had also tried a four weeks' parenthesis with levamisol+spyramycin. Both at enrollement were on paromomycin, and both showed prompt clinical response to rHuGM-CSF(cessation of the diarrhoea in 2 days), but relapsed at therapy discontinuation and required various cycles (4 and 6). At present patient 1 is in very good conditions 8 months after the diagnosis and stopped assuming paromomycin and GM-CSF 3 months ago, cryptosporidiosis having been oradicated. Patient 2 had biliary tract involvement, continued to excrete oocysts and although experiencing reduction of the diarrhoea at every GM-CSF cycle had progressive worsening general conditions and died after 9 months. The general tolerability of the drug was fairly good.

Conclusions: Although the small number of cases enrolled to date does not allow us to draw any definitive conclusions, the results confirm that GM-CSF plays an important role in the immune response, not merely increasing the number of neutrophils and macrophages, but also enhancing their activity against intracellular infectious agents. We mean to continue the study and are planning to extend it to the treatment of drug-resistant MAC infections.

Alterations in cytokine production and cell proliferation of human mononuclear cells induced by a mucin-like (AgC10) from *T. cruzi*

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ABSTRACT. Immunosupression has been reported in the acute phase of *Trypanosoma cruzi* infection but subside during the chronic phase. *In vitro*, living *T. cruzi* induces important alterations in mitogen-activated human T and B lymphocytes and inhibits their capacity to proliferate. Recently, we have characterizated a novel antigen of *T. cruzi* as a mucin-like GPI-glycoprotein containing about 65% carbohydrate (w/w) including sialic acid, termed AgC10. In this work we checked whether AgC10 would induce alterations in the immune response and if it would be associated to cytokine production changes during human mononuclear cells *in vitro* proliferation. A significant inhibition in the level of [^{3}H] thymidine incorporation by PBMC stimulated with the mitogens anti-CD3 or PDB ensued when AgC10 was added to the cultures. This alteration that AgC10 causes in the proliferation of human PBMC was associated to marked disminution in the level of IFN-γ production, a cytokine involved in the recovery from *T. cruzi* infection. Thus, human T lymphocytes stimulated with T-cell-specific mitogen anti-CD3 manifested a suppressed capacity to proliferate when the AgC10 was added. The potential capacity of AgC10 to inhibit human T lymphocyte proliferation and to reduce IFN-γ production verifies the reported differential regulation of IFN-γ production during the course of both natural murine and human infections by *T. cruzi*. However no inhibition of [^{3}H] thymidine incorporation by human macrophages stimulated with the mitogen LPS or without stimulation was observed. Otherwise we detected a higher levels of cytokine IL-1β production in the culture of human macrophages with AgC10 from *T.cruzi*. This increment in the level of IL-1β production by the macrophages was accompanied by AgC10 induced-cellular aggregation. Also the AgC10 induced-aggregation was blocked by monoclonal antibodies against AgC10 and by pre-treating macrophages with azide. The increment in the level of IL-1β production by the macrophages in presence of AgC10 also confirms the evidence that monocytes infected by *T. cruzi* induce higher IL-1β levels.

Effect of cytokines on the infection of CBA mice with *L. amazonesis*.

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Leishmania **infect macrophages of thier veterbrate host, reside in a parsitophorous vacuole within these cells, and then cause a spectrum of diseases dependent in large part on the parasite species. There is ample evidence that immunity to leishmaniasis is dependent on appropriate activation of the cellular immune response. It should however, be expected that different effector responses may be required to limit infection with each of these species. To ascertain the role of cytokines, including IFN-gamma, TNF and IL4, in protection of CBA/J mice against *L. amazonensis*, adoptive transfer experiments were performed using cloned antigen specific CD4+ T cell lines. CD4+ T cell lines reactive to the protective leishmanial antigens GP46 (Sita 5) and P8 (P8/5) were derived. The Sita 5 T cell lines secrete INF-g, IL4 and TNF, while the P8/5 T cell line secretes IFN-g, TNF and no IL4, in response to antigen stimulation. We show that the presence of IL4 has little influence on the course of the infection. The role of the other cytokines are discussed.**

Induction of the Tumor Necrosis Factor α production by human hepatocytes in Hepatitis B Virus infection

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Tumor Necrosis Factor α (TNFα) is a multifunctional cytokine that has an important role in the pathogenesis of inflammation, cachexia and septic shock. Although TNFα is mainly produced by macrophages, there is evidence regarding TNFα production by cells that are not derived from bone marrow. TNFα production by Hepatitis B Virus (HBV) infected human liver has been described by us previously at both mRNA and protein levels. In addition, transfections of HepG2 hepatoblastoma cells with either HBV genome or HBx gene resulted in induction of TNFα expression. Our results demonstrated that HBV infection induces, both in vivo and in vitro, TNFα production in hepatocytes in the absence of other inflammatory stimuli, and indicate that the HBx protein may regulate the expression of this cytokine. Transient cotransfection experiments using HBx-expression vectors and TNFα promoter-derived reporter plasmids indicated a trans-activation of this promoter by the viral protein.

Interferon α (IFNα) is commonly employed in the treatment of patients cronically infected by HBV. This cytokine is known to inhibit HBV replication by interfering the viral transcriptional regulatory elements. We investigated whether the clearance of viral transcripts by treatment with IFNα of the cell line HepG2-2.2.15 (stably transfected with HBV) affected the levels of TNFα expression. We have found a clear correlation between the expresion of viral transcripts and TNFα mRNA.

Cytokine expression in Caprine Arthritis Encephalitis virus (CAEV) infection

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CAE virus is a lentivirus related to Maedi Visna virus, FIV, SIV and HIV. Infection of goats with CAEV does not cause immunodeficiency but leads to chronic inflammation of joints, lung and mammary gland. The progressive chronic inflammation is characterized by infiltrates of macrophages, T and B lymphocytes as well as increasing numbers of plasma cells. Because of its histopathological similarity, CAEV-induced arthritis serves as a model for human rheumatoid arthritis.
Several lines of evidence suggest that CAEV induces a Th2 type immune response but cytokine expression in infected goats has not been studied to date.
Since monocytes/macrophages are the main target of CAEV *in vivo* we are interested in the role of these cells in the pathogenesis of arthritis. We tested the hypothesis that CAEV can alter the pattern of cytokine expression in macrophages. Indeed the expression of several cytokines is dysregulated in infected macrophages *in vitro*. For example IL-12 p40 RNA is similarly downregulated by CAEV as in HIV-infected macrophages.
Additionally we performed time course experiments *in vivo* to follow the spectrum and kinetics of cytokine expression in joints after intracarpal and intravenous infection of goats. *In situ* hybridisation (ISH) experiments showed early and co-localised expression of viral RNA and IFNγ RNA in synovial membranes both of which at later time points became undetectable by ISH. Other cytokines such as TNFα, IL-6 and MCP-1 are expressed throughout the time course. In goats with severe clinical arthritis viral RNA became detectable again in synovial tissue but IFNγ transcripts remained very difficult to detect. Together with the high numbers (> 50%) of plasma cells found in arthritic goats these findings suggest a switch to a strong and non-protective humoral immune response but more studies are needed to proof this hypothesis.

CYTOKINES IN THE IMMUNOPATHOLOGY OF LEPROSY NEUROPATHY
TNF : THE PROGNOSTIC MARKER OF LEPRA REACTION

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Reactions in leprosy consist of severe inflammatory episodes across the spectrum of the disease leading to massive tissue destruction of which nerve damage is the prime component. Neuropathy, the major morbidity factor in leprosy, is the result of host's immune response to the causative organism *Mycobacterium leprae*, which has a predilection for skin and nerves, especially Schwann cells. Pathogenic hypotheses to the host's immune response involves various sets of immunocompetent cells and their secretory products, among which the cytokines. The hypothesis we addressed is to which extent a given set of cytokines produced in excessive amounts is involved in the triggering of tissue lesion(s).

Significantly elevated levels of TNFα, IL-1β, IL-6 and IL-8 in serum of patients with lepra reaction have been reported by our group in cross-sectional studies of patients from Delhi and Dakar. The levels were found high during the episodes of reaction and the LL category. The levels correlated with the severity of nerve damage. The prognostic significance of serum TNFα and IL-1β levels in predicting lepra reaction was reported from this study.

A recent longitudinal study of patients was conducted in a double-blind manner in 160 patients. These patients were followed up clinically over two years period and their circulating TNF levels were measured by ELISA (Medgenix, Fleurus, Belgium) at periodic intervals. At the end of the study the codes were broken and the levels were correlated with their neurological, clinical and treatment status. Patients with TNF level >60 pg/ml developed subsequent reaction and leprosy neuropathy. The Predictive value of the serum TNF level over 60 pg/ml was 97.2%. Patients with very high levels of serum TNF at the first bleed point manifest with permanent neurological deficit in course of their therapy irrespective of the time gap. Neurological deficit was found up to 9 months after initial diagnosis confirming its use as a prognostic marker for determining the patient at risk. The correlation of circulating TNF level with the conventional therapy of steroid and thalidomide has also been demonstrated.

We have investigated the mechanism of neuropathy by studying various cytokines and adhesion molecules at lesional level in the nerve biopsies of leprosy patients using immunohistochemistry. Elevated expression of TNFα in the granuloma areas was observed implying its direct role in the immunopathology of nerve damage. There was strong immunostaning for ICAM and LFA-1. The lesions were infiltrated with activated T cells and macrophages as evidenced by CD3, CD8 and CD68 staining. Lesional cells expressed HLA-DR suggesting activation by IFN-γ. Thus, the pathology seen in the form of axonal degeneration and segmental demyelination in nerves of patients are mediated by TNFα and other adhesion molecules like LFA-1 and ICAM-1.

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Title : SOLUBLE IL-6 RECEPTOR AND IL-6 LEVELS IN SERUM AND SYNOVIAL FLUIS OF PATIENTS WITH DIFFERENT ARTHROPATHIES.

Objective. We studied IL-6 and soluble IL-6 receptor (sIL-6R) in serum and synovial fluid (SF) to investigate its role in different arthropathies.

Methods. IL-6 was measured by ELISA and bioassay and sIL-6R by ELISA, in 110 sera and 73 SF samples from 49 patients with rheumatoid arthritis (RA), 20 with crystal deposition disease (CDD), 17 with osteoarthritis (OA), 24 with other arthritis (OIA) and 100 controls. In all patients, disease activity was assessed by laboratory parameters (ESR and CRP) and in patients with RA and OIA pain, tender and swollen joints, Ritchie index and morning stiffness was also evaluated. In SF total leucocyte count (TLC) was determined.

Results. There was a good correlation between IL-6 ELISA and bioassay levels both in serum ($r=0.62$, $p=0.0001$) and in SF ($r=0.72$, $p=0.0001$). Serum IL-6 was detected only in patients with inflammatory arthritis and SF IL-6 was detected in all patient groups. Serum IL-6 levels correlated with swollen joints ($r=0.35$, $p=0.05$), ESR ($r=0.46$, $p=0.001$) and CRP ($r=0.46$, $p=0.001$) in RA, and with CRP ($r=0.89$, $p=0.0001$) in CDD. SF IL-6 correlated with ESR ($r=0.54$, $p=0.007$) and CRP (0.42, $p=0.04$) in RA, with SF TLC ($r=0.61$, $p=0.004$) in CDD and with SF TLC ($r=0.61$, $p=0.09$) in OA. No correlations were found in the OIA group. sIL-6R was found in significant amounts in sera of controls and was also detectable in all patient groups whilst only in the RA group sIL-6R levels were significantly increased ($p=0.05$). SF sIL-6R levels were lower than in serum in all patient groups. No correlations were found between sIL-6R and IL-6 or between sIL-6R and disease activity parameters in any group.

Conclusion. Our results suggest that unlike IL-6, sIL-6R is not produced at the site of inflammation and is not related to clinical or biological parameters of disease activity. Only in RA both IL-6 and sIL-6R levels are increased suggesting that sIL-6R may aid systemic effects of IL-6.

TNF-ACTIVATED ENDOTHELIAL CELLS EXERT A CYTOTOXIC EFFECT ON TUMOR CELLS BY PRODUCTION OF NITRIC OXIDE

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We have previously shown that the arrest or regression of mouse liver metastases formed by *lacZ* -transduced ESbL T lymphoma cells (ESbL-*lacZ*) is associated with the stimulation of nitric oxide (NO) production by liver endothelial cells *in situ*. NO is generated in cytokine-activated macrophages and vascular endothelial cells from the oxidation of L-arginine by inducible NO synthase (iNOS) and is considered as an effector molecule of anti-tumor cytotoxicity. Here we studied *in vitro* the production of NO and the cytotoxicity against ESbL-*lacZ* lymphoma target cells exerted by activated bovine endothelial cells (BEC). It was found that the overnight co-culture of BEC with human TNF-α (final concentration - 1 nM) caused a stimulation of iNOS activity and an increase of NO synthesis by endothelial cells. Incubation of activated BEC with lymphoma cells (ratio 50:1, 25:1 or 10:1) led to the death of the latter cells as evidenced by staining with propidium iodide and FACS analysis. This anti-tumor cytotoxicity was time-dependent and appeared as early as 1h after co-culture, reaching a maximal level after 4h followed by a reduction of the cytotoxicity after 24 h of incubation. Non-activated BEC, which are not able to produce NO through iNOS dependent mechanism, showed a substantially lower level of cytotoxicity against lymphoma target cells. We next directly tested whether ESbL-*lacZ* cells are sensitive to the toxic effect of NO. Treatment of the cells with glycerol trinitrate (GTN) which is known to produce NO in the incubation medium, resulted in a profound cytotoxic effect against lymphoma cells. The level of cytotoxicity correlated with the concentration of NO in the medium. The possible role of NO in endothelial cells mediated anti-tumor reactivity *in vitro* and *in vivo* will be discussed.

REGULATION OF THE EARLY CYTOKINE RESPONSE TO MALARIAL INFECTION

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Malaria fever is caused by the release of TNF and other cytokines, produced by monocytes and macrophages in response to toxins released by rupturing schizonts. Excessive TNF production predisposes to fatal complications including cerebral malaria.

This study aims to define critical events in the regulation of the early response to malarial toxins in the human monocyte cell line MonoMac6. We found that TNF mRNA was expressed within 30 min of stimulation by malarial toxin. The response was completely blocked by actinomycin D but not by cyclohexamide, indicating that it was due to transcriptional activation by regulatory factors already present in the cell. Expression of mRNA for IL-12p40, IL-6 and ICAM-1 was observed within 2 hrs but this could be inhibited by cyclohexamide, indicating dependence on protein synthesis. Repeated stimulation of MonoMac6 cells with malaria toxin led to the induction of tolerance.

Work is in progress to define the regulatory pathways involved in transcriptional activation of the TNF gene with malarial toxin.

List of Invited Speakers

Workshop on

CYTOKINES IN INFECTIOUS DISEASES

List of Invited Speakers

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