P325 CELLULAR IMMUNE RESPONSE IN HUMAN DILATED CARDIOMYOPATHY

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With regard to the hypothesis of the human dilated cardiomyopathy (DCMP) as partly virusinduced autoimmune disease we investigated the monocyte and T-cell activation.

In 7 responders out of 29 patients with idiopathic DCMP the serum levels of the monocyte cytokine *Tumor necrosis Factor Alpha (TNF)* was increased two- to fourty-fold. These extreme values in some individuals resulted in a significantly higher total count in relation to the normal donors, although the vast majority of the DCMP patients showed a normal TNF secretion (mean percentage is 520 % of the serum levels in 10 normal subjects). In contrast, *Interleukin 1 Alpha (IL-1$^{\alpha}$)* showed no significant difference between the normal controls and the DCMP patients, neither the endogenous secretion into the sera nor the in vitro production by the peripheral blood lymphocytes (PBL) stimulated by lipopolysaccharide.

Investigating the T-cellular immune reponse, we measured significantly higher values of the *soluble Interleukin 2 receptor (sIL-2R)* in the serum of the DCMP patients compared to the normal donors (112.3±49.3 versus 68±48 pM, $p<0.025$). A similar trend was detected when we measured *in vitro the blastogenic reponse of PBL* to unspecific mitogens and to IL-2 as well as the levels of the sIL-2R and IL-2 in the *supernatants* of mitogen-stimulated PBL. A specific T-cell response to the pathogenetically relevant Coxsackievirus B3 was detected in 6 of 12 seronegative DCMP individuals, thus more sensitive than the virus-serology.

In conclusion, we found that DCMP patients had an activated T-cell and monocyte/macrophage system with an elevated specific and unspecific blastogenic response of T-lymphocytes and an increase of the sIL-2R and TNF. This suggests autoimmune mechanisms against myosin and/or viral antigen. We regard other virusinduced autoimmune diseases (i.e. chronic hepatitis B) and the allograft rejection as pathogenetic models.

P326 MODULATION OF THE EFFECTOR CELL-TARGET CELL INTERACTION BY ANTIBODIES TO CD2 AND CD11a/CD18

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Our previous studies have shown that the extent of cell-to-cell contact between effector and target cell is positively related to the lytic potential of both natural killer (NK) and lymphokine activated killer (LAK) cells.

To verify whether a large contact area is necessary to trigger effector cell activation, we evaluated the effect of antibodies to CD11a/CD18 (LFA1) and CD2 on conjugate formation. The interaction pattern between NK or LAK cells and tumor targets with different susceptibilities, such as K562 and HL60R cells, was studied by scanning electron microscopy (SEM) and flow cytometry. Anti-CD11a/CD18 and anti-CD2 antibodies did not inhibit the conjugate-forming ability of the effector cells. In contrast, the antibodies produced a remarkable inhibition of the lytic function accompanied by a significant reduction in the contact area (the so-called "closed chamber") between effector and target cells. A quantitative analysis on SEM images showed that in control cell pairs the average surface of contact was about 10 square micrometers versus about 3 square micrometers in conjugates formed in the presence of antibodies to CD11a/CD18 and *CD2*.

These observations indicate that antibodies to CD11a/CD18 and CD2 reduce the extent of target/effector interactions which could be responsible for a decreased level of effector cell activation.

P327 NEUTROPHIL CR1 RECEPTOR: RELATIONSHIP BETWEEN EXPRESSION LEVEL AND CYTOTOXICITY IN HEALTHY AND CHRONIC MYELOID LEUKEMIA.

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The evaluation of complement receptor CR1 on peripheral blood neutrophils from patients with chronic myeloid leukemia (CML) showed that 7 out of 22 had a marked reduction in CR1 expression and neutrophil stimulation with PMA did not change the receptor extent, while in healthy cells it was increase up to two folds. In addition, functional analyses of CML patients'neutrophils with low CR1 expression showed a dramatic reduction in cytolytic activity against K562 target cells, suggesting a relationship between the expression of CR1 and tumoricidal activity. CML patients treated with α-interferon therapy showed a progressive increase in CR1 expression together with a concomitant enhancement in cytotoxicity. This could indicate that CR1 receptor is modulated by α-interferon in vivo.

The involvement of CR1 in neutrophil-mediated lysis is consistent with the complete lack of the cytolysis against tumour target following the receptor neutralization by anti-CR1 monoclonal antibodies.

P328 INVOLVMENT OF RETINOIC ACID ON THYMOCYTES DIFFERENTIATION

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Retinoic acid (RA) is known to be a powerful differentiating agent, most likely interacting with the chromatine structure after binding to a defined nuclear receptor and then promoting a gene trascription cascade. Both the binding to the cellular membrane and the cytoplasmic transport are still poorly understood. A number of studies has been done using human and murine myeloid cell lines . Based on them we decided to investigate whether this molecule could play a role during the human intrathymic T cell differentiation. Unfractionated thymocytes were obtained from patients (< 5 years old) undergoing corrective cardiac surgery . A T cell line, named RPMI 8402, resembling the features of the stage I thymocytes ($CD2^+$, $CD3^-$ $CD4^-$ $CD8^-$ $CD1^-$) at the membrane and molecular level ,was used to overcame the low recovery of that immature population. All cells were coltured in RPMI 1640 10% FCS in the presence of 10^{-6} M RA (Sigma type XX: all trans) and Facs analysis performed at day 0 and 6. The RA is able to mantain the unfractionated thymocytes 85% viablility (by Trypan Bleu exclusion) in the absence of Dna synthesys measured by ^{3}H-thymidine uptake and to strongly increase the CD3, CD4 and CD25 membrane expression .From the same population driven to proliferate by RA, PMA (1 ng/ml) and recombinant IL2, give rise to two subsets : small thymocytes bearing the phenotype of the cell treated with RA alone (60%) and large blasts showing the more mature features of the 3th stage ($CD1^-$, $CD4^+$ or $CD8^+$) . The only CD4 mRNA transcription and surface expression was elicited in RPMI 8402 cell line by RA tretment alone . Taken toghether , although preliminar, these data suggest the retinoic acid may play a role during T cell lineage differentiation.