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FK 506 AND RAPAMYCIN EXERT DIFFERENT EFFECTS ON THE PROLIFERATIVE RESPONSE OF MONONUCLEAR PHAGOCYTES TO MACROPHAGE COLONY-STIMULATING FACTOR MH Cooper, SH Gregory, AW Thomson, JJ Fung, TE Starzl, EJ Wing, Departments of Surgery and Medicine, University of Pittsburgh School of Medicine, Pittsburgh, PA, USA 15261

Studies involving the immunosuppressant FK 506 and its structural analogue rapamycin have focused largely on T lymphocytes. Comparatively little is known about effects of these drugs on biological activities of other immune cell populations. Studies were undertaken to determine the effect of FK 506 and rapamycin on the proliferative response of mononuclear phagocytes (MNP) to macrophage-colony stimulating factor (M-CSF). Murine bone marrow-derived MNP were subcultured in the presence of various concentrations of FK 506 or rapamycin and 0,1% and 10% L cell-conditioned medium, an exogenous source of M-CSF. After 3 days, the proliferation and survival of cells was assessed by their ability to metabolise the tetrazolium salt, MTT. Rapamycin treatment resulted in diminished capacity of MNP to metabolise MTT in the presence or absence of M-CSF. In contrast, FK 506 had little effect on the proliferation of cells cultured in the presence of optimal concentrations of M-CSF and increased the metabolism of MTT by cells cultured in the absence of M-CSF. Such findings may have important clinical implications with regard to the ability of patients receiving these immunosuppressants to generate a functional MNP population.

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THE BIOLOGY OF HUMAN AND VIRAL IL-10. R. de Waal Malefyt, H. Yssel, M.-G. Roncarolo, B. Bennett, H. Spits and J.E. de Vries (Spon: A. Zlotnik).
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h-IL-10 is produced by most $CD4^+$ T cells, a proportion of $CD8^+$ T cells, B cells and monocytes. IL-10 has strong homology with an open reading frame in the Epstein-Barr virus, BCRF1. BCRF1 was expressed and its product was designated viral (v)-IL-10. Biological characterization of rec h-IL-10 and v-IL-10 showed that they blocked the synthesis of IFN-γ, GM-CSF, TNFα, TNFβ produced by T cells following polyclonal activation. In addition, IL-10 and v-IL-10 strongly prevented antigen specific proliferation of $CD4^+$ T cells and $CD4^+$ T cell clones, when monocytes were used as APC. This inhibitory effect could not be neutralized by exogenous IL-2 and was due to the strong downregulatory effects of IL-10 and v-IL-10 on class II MHC expression on monocytes, which resulted in a strong reduction in T cell activation as judged by induction of Ca^{2+} fluxes. IL-10 and v-IL-10 had also direct anti-proliferative effects on T cell clones activated via CD2 or by PHA in the absence of monocytes, that were due to inhibition of IL-2 production and could be neutralized by exogeneous IL-2. Furthermore, IL-10 and v-IL-10 strongly blocked IL-1α,β, IL-6, IL-8, GM-CSF and TNFα production by activated monocytes, which occurred at the transcription level. Interestingly, IL-10 and v-IL-10 blocked IL-10 mRNA expression in monocytes, indicating that IL-10 has autoregulatory effects. Collectively these data demonstrate that IL-10 and v-IL-10 because of their downregulatory effects on class II MHC expression on monocytes, prevention of antigen specific T cell proliferation and inhibition of proinflammatory cytokine production are strong immunosuppressants.

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INTERFERON-γ RECEPTOR/IMMUNOGLOBULIN HYBRID MOLECULES AS INHIBITORS OF IFNγ FUNCTION

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IFNγ is a lymphokine produced by activated T and NK cells that plays a major role in the course of the immune response. Its receptor is ubiquitously expressed on the cell surface. In order to closely examine the role of the IFNγ in the body, inhibitor molecules were prepared which block its binding to the receptor. We constructed hybrid molecules comprising the ligand-binding part of the IFNγ receptor (extracellular portion) and constant regions of immunoglobulin chains. Such molecules retain the high affinity for IFNγ and high stability of immunoglobulins (as evidenced by the longer serum half-life than the similar molecule that has only extracellular part of the receptor). These inhibitors are used for pharmacokinetic studies after injections in mice and later on for the investigation of their therapeutical value in the treatment of some autoimmune diseases, chronic inflamations and allograft rejection.

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ROLE OF SPECIFIC IL-1β AND IL-1ra RESIDUES ANALYZED BY SITE DIRECTED MUTAGENESIS. S.P. Eisenberg, R.J. Evans, J.D. Childs, B.J. Brandhuber, and R.C. Thompson. Synergen, Inc., 1885 33 Street, Boulder, Colorado 80301 USA

Site directed mutagenesis of IL-1β and IL-1ra was used to study the role of specific residues on receptor affinity and agonist activity. R11, H30, and D145 of IL-1β and W16, Y34, and K145 (the corresponding residues) in IL-1ra were changed. Two of our IL-1β muteins, H30 changed to an A (β-H30A) and β-H30G, showed >20-fold reduced affinity for the IL-1 receptor (IL-1R). Of the IL-1β muteins that have normal or near normal affinity, β-R11G and β-D145K showed greatly reduced agonist activity in long term assays, consistent with previous reports by others. However, of these 2 muteins, only β-D145K inhibited IL-1 in these assays, raising the question of whether β-R11G is stable. The other 2 IL-1β muteins that we studied, β-R11A and β-D145G, showed normal IL-1R affinity and agonist activity. IL-1ra mutagenesis gave 3 muteins, ra-W16G, ra-W16R and ra-Y34G, with ≈4-6 fold reduced affinity for IL-1R. These muteins were also significantly reduced in IL-1 inhibitory activity. The remaining three IL-1ra muteins, ra-Y34H, ra-K145D and ra-K145G, are normal in affinity for IL-1R. None of the IL-1ra muteins had any agonist activity in IL-1 bioassays utilizing human cells. However, ra-K145D did exhibit a small but significant agonist activity on cells expressing the murine IL-1R, as shown previously by Ju *et al.* In summary, residue 145 of either IL-1β or IL-1ra is important in the ligand's agonist activity, and β-H30, ra-Y34 and ra-W16 are important for IL-1R binding. The role of β-R11 in IL-1 action is currently under study.

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HUMANISED ANTIBODIES FOR ANTI-TNF THERAPY. Spencer Emtage and Mark Bodmer. Celltech Ltd, Slough SL1 4EN, Berks, UK.

Overproduction of TNF has been implicated in the pathogenesis of a variety of clinically important conditions. Monoclonal antibodies which neutralise TNF may have a role in therapy. Murine anti-TNF has been shown to be immunogenic in man. In an attempt to avoid this problem, a genetically engineered anti-TNF antibody has been produced. Three issues were addressed to make a reagent suitable for clinical development. Firstly, a potent neutralising murine antibody was selected on the basis of its ability to block TNF in vitro in both cytotoxicity and endothelial cell activation assays. It was also shown to block huRecTNF induced pyrexia in rabbits. Secondly, a CDR-grafted version of the antibody was made which substantially retains the affinity and biological activity of the murine parent. Thirdly, it was established that the anti-TNF in a human IgG4 background retains activity in the rabbit pyrexia model. This novel, humanised anti-TNF reagent should permit a full evaluation of the potential for TNF-neutralising therapy in the clinical.

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PRODUCTION OF IL-1 AND IL-1 RECEPTOR ANTAGONIST BY HUMAN ALVEOLAR MACROPHAGES. B Galve-de Rochemonteix, C. Baumberger, *L.P. Nicod and J.-M. Dayer
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We have previously shown that human alveolar macrophages (AM) produce a specific 20-25 kD IL-1 inhibitor blocking the activities of IL-1α and IL-1β. It competitively binds to the IL-1 receptor on murine EL4-6. We present evidence that the IL-1 inhibitor is identical with the IL-1 receptor antagonist (IL-1ra) cloned from IgG-stimulated monocytes. Western blot analysis performed on the 48 h AM supernatant using a polyclonal rabbit antibody against rhIL-1ra (gift from Synergen, Boulder) showed a band at 20kD which is slightly higher than that obtained against rhIL-1ra. The results after Northern blot analysis performed with the oligonucleotide IL-1ra probe (gift from Synergen, Boulder) on RNA from AM stimulated with PMA are shown below:

	S I 8h/0	S I. 8h/15 min	S I. 8h/30 min	S I. 8h/1h	S I. 8h/3h
IL-1 β	undetected	9.8	1.8	1.1	0.55
IL-1 α	undetected	12.3	3.8	2.9	1.9
IL-1ra	undetected	undetected	undetected	undetected	3.0

IL-1ra is not immediately expressed after bronchoalveolar lavage (time 0) but appeared at 3 h and 8 h. IL-1 α and β mRNA were expressed earlier at 15 min. These results indicate that in vitro AM synthesize and secrete IL-1ra. During inflammatory processes in the lung this release may be important in modifying the effects of IL-1α and β.