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IMMUNOMODULATION BY HISTAMINE,SEROTONINE AND DOPAMINE

P.Leskovar, N.Mattele, T.Reithmeier, F.Siegmund, and H.Hesch
Immunological-biochemical Research Laboratory, School of Medicine, University (TU) Munich;Klinikum r.d.I.,Ismaningerstr.22, D-81675 München

We tested a number of physiological and synthetic ligands of those receptors which directly modulate the intracellular level of cyclic nucleotides (cAMP, cGMP) and herewith the activity of the corresponding protein kinases (cAMP-dependent PKA and cGMP-dependent PKG). Of special interest was the question whether the immunosuppression, induced by different cAMP-elevating ligands and their combinations could be counteracted or even reversed to immunostimulation by the post-incubation of immunosuppressive cells with blockers of these receptors and/or agonists of cAMP-depressing, i.e. Gi-coupled receptors. Here, we discussed the physiological „first messengers" histamine, seratonine and dopamine.
We used the following protocol: First, lymphocytes from person A were incubated for 2 days with different test substances, washed out and divided in 2 portions, one being post-incubated for 18h with various antagonists of the Gs-coupled receptors or agonists of Gi-coupled receptor subtypes, respectively, and the other one serving as control (without addition of test substances). The next step was the evaluation of the immunosuppressive or immunostimulating effect, respectively, of so pretreated cells on an alloantigen- or lectin-activated standard cell suspension.
Our results show that ligands of both the immunological and the non-immunological receptors influence cell function via modulation of second messengers and that this action is additive. So, the 16% histamine-mediated immune suppression could be reversed to a 20% net immunostimulation. Similarly, the 38% suppression of PBLs by serotonine in the PHA-assay could be reversed to a 27% net stimulation by the selective antagonist cyproheptadine. The corresponding values for the MLC-assay were a 36% immune suppression (by seratonine) versus a 9% immunostimulation (by cyproheptadine).

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INFLUENCE OF SOME INTRACELLULAR cAMP-LEVEL INCREASING SUBSTANCES (a) ON RESTING AND (b) ON PROLIFERATING IMMUNOCYTES

P.Leskovar, T.Reithmeier, F.Siegmund, and N.Mattele
Immunological-biochemical Research Laboratory, School of Medicine, University (TU) Munich; Ismaningerstr.22, D-81675 München

The aim of our study was a direct comparison of the immunosuppressive effect of some test substances which increase the cytosolic cAMP-level by the ligation of receptors, coupled to the Gs-subunit of the GTP-dependent protein (G protein), on the surface (a) of resting and (b) of rapidly proliferating immune cells, respectively. (a) The effect of these test substances on immunocompetent cells in the resting state (Go phase) was measured as follows: PBLs from person 1 were incubated for 48h in the presence or absence of test substances, thoroughly washed and used in 2 assays, the classical „one-way-MLC" and the standard „lectin (PHA)-proliferation assay". For the MLC, the 48h-preincubated PBLs were first mixed (1:1) with fresh PBLs of the same person 1, added to an equal number of mitomycinC-pretreated PBLs of person 2 and coincubated for 5 days; their activity was determined by the WST-1 test. The PHA proliferation assay consisted of a 2d-postincubation of test substances-pretreated PBLs of person 1 with fresh PBLs of the same person 1 (ratio 1:1) in the presence of 1ug/ml PHA. (b) The effect of cAMP-increasing test substances on proliferating immunocytes was measured by a two-step-MLC: in the first MLC, PBLs of person 1 and person 2 were coincubated in the presence or absence of test substances for 5 days, washed, mixed (1:1) with fresh PBLs of person 1 and added to mitomycinC-inactivated PBLs of person 2. After a further 5 d-coincubation, the cell activity was determined by the WST-1 assay.
Our results show a significant immune suppression by cAMP - increasing ligands, both in resting and proliferating immunocytes. It seems that resting PBLs, including non-primed alloreactive T subpopulations and naive lectin-inducible T subclones become „programmed" to direct the differentiation of precursor T cells towards the Th2 or Ts subset, when preincubated with cAMP-inducing ligands. The introduction of the two-step-MLC allows the generation of allospecific Th2 and Ts cells which are of special interest in BMT.

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IMMUNE REGULATION VIA NON-IMMUNOLOGICAL RECEPTORS ON IMMUNOCOMPETENT CELLS: STRONG IMMUNOSUPPRESSION, INDUCED BY ADENOSINE AND EPINEPHRINE

P.Leskovar, F.Siegmund, N.Mattele, T.Reithmeier, and H.Hesch
Immunological-biochemical Research Laboratory, School of Medicine,University(TU)Munich; Klinikum r.d.I.,Ismaningerstr.22, D-81675 München

The basic idea of our experiments was the assumption that both, the immunorelevant and the non-immunological signals contribute to the intracellular level of second messengers, such as cAMP, cGMP or Ca_i^{++} and that the „filtering-out" of the excessive background signals could restore the susceptibility of hypoergic or anergic immunocytes for new antigen signals. As known, agonists of both the immunorelevant and non-relevant Gs-coupled receptors on immunocytes contribute (via stimulation of the receptor-associated adenylic cyclase) to the elevation of the cytosolic cAMP-level,which results in the depression of the cellular function and - if persistent - in a generalized immunosuppression. The antagonists of Gs-coupled receptors and/or the agonists of Gi-coupled subtypes of the same receptors, on the other hand, are able to counteract this immunosuppressive effect by inhibiting the receptor-coupled adenylic cyclase.
To test our hypothesis, anticipating an active role of non-immunological agonists, such as adenosine and epinephrine on the net activity of immunocompetent cells, we carried out the following experiments: The lymphocytes (PBLs) from person A were first incubated in the presence or absence of different physiologic and synthetic agonists and antagonists of Gs- and Gi-coupled receptor subtypes. Two days later, the so pretreated cells were either (a) mixed (1:1) with fresh PBLs of the same person A and coincubated for 5 days with mitomycin C-pretreated PBLs of person B (one-way-MLC) or (b) stimulated (for 2 days) with the mitogenic lectin PHA.
We found that adenosine and epinephrine per se and especially if combined with ConA lead to a strong Ts-generation and that a variety of substances, e.g. the blockers of the A2/P1 purinergic and ß-adrenergic receptor prevent or even reverse the Ts generation.

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SUPPRESSOR T CELL (Ts) AND CYTOTOXIC T CELL (Tc/CTL): THE SAME CELL IN TWO FUNCTIONAL STATES, DIFFERING (a) IN THE ACTIVATION OF PC vs. PI- DEPENDENT PLC UPON CD3/Ti-LIGATION, AND (b) IN THE DEPENDENCE ON EXTRACELLULAR Ca^{++}- SOURCE?

P.Leskovar, F.Siegmund, T.Reithmeier, and N.Mattele
Immunological-biochemical Research Laboratory, School of Medicine, University (TU) Munich; Klinikum r.d.I., Ismaningerstr.22, D-81675 München

According to our hypothesis, the Ts and the Tc cell which cannot be distinguished phenotypically represent the same cell in 2 functional states. We suggest that the main difference between both functional subsets is (a) the activation of the PC (phosphatidyl choline) dependent PLC (phospholipase C) in the Ts cell, as opposed to the activation of the PI (phosphatidyl inositol) dependent PLC in the Tc cell upon CD3/Ti ligation, and (b) the critical dependence on the extracellular Ca^{++}- source in Ts but not in Tc cells. Interestingly, IFN gamma, TNFalpha and other differentiation-inducing cytokines also stimulate the PC, not the PI-dependent PLC, the IFN-R resembling most closely the receptor for the type 2 cytokine IL-10.
Our own experiments aimed at the demonstration of a simultaneous immunosuppressive and cytotoxic activity of T8 cells, more precisely, of T8-containig peripheral blood lymphocytes (PBLs).
The immunosuppressive effect was quantified by the inhibited proliferation of a standard cell suspension, exposed to alloantigen and lectin-stimulus, respectively. The cytotoxicity assay was based on the determination of LDH (lactate dehydrogenase)-release from allogeneic target blasts, pregenerated by a 3 day-incubation with PHA.
We show that the same, T8 cells containing PBLs are able both to lyse target cells and to suppress cell proliferation. This PBL proliferation is additionally supported by Ca antagonists which seem to depress selectively the Th2 and Ts subclones. To our understanding, an increased cytosolic cAMP level downregulates the activity of the PI dependent PLC by the PKA-mediated phosphorylation of serine at the position 1248 of PLC gamma1.