## 35 INTERACTION OF LPS AND ConA IN HUMAN LYMPHOCYTE ACTIVATION. I: SYNERGISTIC EFFECT ON IgM PRODUCTION.

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While LPS is the most potent B cell activator in mice, in man induction of B-cell differentiation has been reported only under stringent culture conditions.*In 7-day cultures of $10^5$ peripheral blood lymphocytes in 0.2 ml of RPMI containing 10 % FCS in flat-bottomed culture plates, LPS over a wide range of concentrations (0.1 to 250/ug/ml) does not stimulate detectable IgM production while ConA at optimal (6 ug/ml) and suboptimal (0.6 ug/ml) mitogenic concentrations induces synthesis of only low amounts of IgM (250-600 ng/ml). However, when LPS is added to ConA-stimulated cultures a striking dose-dependent enhancement of IgM production, maximum (1200-2100 ng/ml) with 10 ug/ml of LPS is evident. A similar enhancing effect of LPS on IgM production with a mean increase of 100 % was observed also in PWM-stimulated cultures. Studies are now underway to clarify the cellular basis underlying this phenomenon.

* T. Kunori, O. Ringdén, E. Möller. Scand.J.Immunol., 8, 451, 1978.

## 36 INTERACTION OF LPS AND ConA IN HUMAN LYMPHOCYTE ACTIVATION. II: EFFECT ON CELL PROLIFERATION.

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In order to evaluate the cellular basis of the synergistic effect of LPS and ConA on in vitro IgM production their effect on cell proliferation was studied under identical experimental conditions in normal subjects and in patients with X-linked agammaglobulinaemia (XLA). While cell viability was not affected by any dose of LPS, increasing doses of LPS progressively reduced thymidine incorporation on day 3 in ConA stimulated cultures from 10 normal subjects; on day 5 inhibition of DNA synthesis was still present except in those doses of LPS eliciting maximum IgM production where a synergistic effect became evident. In 4 patients with X-LA synthesis of IgM was not induced by any combination of PWM, ConA or LPS. The pattern of thymidine incorporation was however similar to that observed in normal controls indicating that the inhibitory effect of LPS on cell proliferation is not dependent on the presence of functional B cells.

## 37 ConA-INDUCED THYMUS-DEPENDENT Ig PRODUCTION BY HUMAN B LYMPHOCYTES IN VITRO.

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While there is good evidence that PWM activates helper T cells for Ig production, the effect of ConA is usually that of suppression. T and B cells were separated from tonsils by E-rosetting and cultured in the presence of ConA for 7 days in 1 ml RPMI containing 10 % FCS in round-bottomed culture tubes. ConA induced no IgM synthesis in cultures of purified B cells and only marginal IgM production (360 ng/ml) in unseparated tonsil cells. However, when increasing numbers of purified T cells were added to $0.5 \times 10^6$ purified B cells, IgM production (1500 ng/ml) was elicited only at low T/B cell ratios (1/5). When T cells were preirradiated with 2500 R increased IgM production was also observed but only at a T/B ratio of 1. In all these conditions ConA consistently induced IgM production only at an optimal mitogenetic dose (6/ug/ml). These findings show that ConA can stimulate helper T cells and that low T cell numbers or T cell irradiation facilitates helper function perhaps by preventing the emergence of suppressor effects.

## 38 FAILURE OF INHIBITION OF LYMPHOCYTE PROLIFERATION BY HUMAN NEWBORN MONOCYTES CORRELATED WITH A LOW PGE2 SECRETION

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We showed that an excess of autologous or allogeneic monocytes, isolated from adult blood, added to mitogen or antigen-induced lymphocyte cultures inhibited their proliferative responses. Similarly, adult monocytes reduced the generation of Pokeweed mitogen-induced plasma cells. These suppressive effects were both mostly reverted by adjunction of Indomethacin ($5.10^{-7}$M). In contrast, newborn (NB) monocytes did not exert any effect on proliferation and generation of plasma cells. Adherent NB monocytes were shown to secrete 10 times less prostaglandin E2 (PGE2) during a 24hrs incubation than adult monocytes. (2.300 vs 24 000 pg/m/$10^6$ monocytes). After activation by zymosan, NB monocytes secreted 6 to 10 times more PGE2. The same effect was obtained by incubation in supernatant of allogeneic leukocyte cultures and NB monocytes became able to suppress T and B in vitro functions. Also monocytes from late pregnant women did not suppress T lymphocyte proliferation. Our observations suggest that absence of suppressive effect of NB monocytes is due to an inhibition of PGE2 secretion rather than to an intrinsic immaturity. This phenomenon, which seems correlated with an excess of suppressor activity exerted by NB T lymphocytes on B lymphocyte maturation might play a role on immune responses in neonatal period.

## 39 THE EFFECT OF THF IN IMMUNOSUPPRESSED CHILDREN WITH LYMPHO PROLIFERATIVE DISEASES.

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THF, a partially purified Thymic Hormone, was shown to restore the impaired cell mediated immunity (CMI) and reduce the visceral complications in 4 children with acute leukemia and generalized varicella. Twelve children with acute lymphocytic leukemia (ALL) or lymphoma were treated with THF for generalized varicella infection associated with impaired CMI. CMI parameters were studied before, during and post THF therapy. It was found to increase significantly (two- to ten-fold) the number of peripheral blood lymphocytes, E-rosettes, response to ConA, PHA and intracellular cAMP in 11 children. The number of lymphocytes and E-rosettes increased from 700 to 6000 and from 250 to 5500 per Cu.mm respectively. All children recovered from the varicella infection, including two with severe bilateral pneumonitis, and all of them developed antibodies to V-Z virus. Similar results of the above mentioned CMI parameters were obtained in 6 additional immunosuppressed children with ALL & lymphoma, 4 of whom had severe pneumonitis. THF restored positive GVHR and skin tests in 8 out of 10 and 4 out of 9 children studied respectively. These observations suggest that THF may restore the impaired CMI in children with ALL & lymphoma and may be valuable as supportive therapy of severe viral infections.

## 40 THE ROLE OF VITAMIN $B_{12}$ AND ITS TRANSPORT GLOBULINS IN GRANULOCYTE BACTERIAL KILLING

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Granulocytes from a 6 year old boy with congenital transcobalamin II deficiency were found to have abnormally low antibacterial activity against Staphylococcus aureus and very low intracellular levels of the cobalamin coenzymes. Transfusion of normal plasma supplemented with hydroxocobalamin temporarily restored granulocyte bactericidal activity to normal and increased cellular levels of the cobalamin coenzymes. Granulocyte function was also temporarily restored by oral leucovorin. The defect appears to be causally related to the patient's TC II deficiency and indirectly to a deficiency of cobalamin and folate coenzymes.