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KINETIC ANALYSIS OF SOLID PHASE C3 FRAGMENTS DEPOSITED ON CROSS-LINKED DEXTRAN GEL BEADS. M. Amin Arnaout, Chester A. Alper and Fred S. Rosen. Harvard Medical School, Center for Blood Research, Children's Hospital Medical Center, Division of Immunology, Boston.

Upon activation of the third component of complement (C3) by the classical or alternative pathway convertases, an opsonically active form of C3 gets firmly bound to a wide variety of surfaces. To analyze the forms of solid phase C3, dextran gel beads (675,superfine) were incubated with normal human serum in veronal-buffered saline 5 mM Mg^{++}, 10 mM ethylene glycol tetraacetic acid (EGTA) for timed intervals, and samples washed with phosphate-buffered saline (200 x volume) at room temperature. Gel beads were solubilized with Dextranase, and solutions analyzed on sodium dodecyl sulfate gels in unreduced and reduced (2-Mercaptoethanol) form. C3 fragments accounted for more than 90% of the total proteins present. A 175,000 M.W. fragment was the predominant species at 5', 10', 15', 20' and 30 minute intervals, and was maximum at 15'. Other fragments were detected as early as 5' (155,000 M.W.), 10' (138,000 and 23,000), 15' (110,000), and at 60' only these and not the 175,000 M.W. fragment were detected.

Solubilization of dextran gel beads with Dextranase appears to be an ideal vehicle for the analysis of solid phase activation and degradation of C3 and the relationship of the deposited fragments to these various biological activities including opsonization and immune adherence.

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RESISTANCE OF CULTURED CHORIOCARCINOMA CELLS TO CYTOTOXIC LYMPHOCYTES, Charles S. August, Sheila T. Cox, Michael A. Naughton, University of Colorado Medical Center, Denver.

Cultured choriocarcinoma cells (BeWo) exist which share many of the morphologic and biosynthetic properties of normal human trophoblasts.In an attempt to develop a model for the immunologic relationship between mother and fetus, we mixed BeWo cells with mitogen-activated cytotoxic lymphocytes in vitro. Microscopic observations revealed that BeWo cells exhibited a marked resistance to the cytolytic effects of the activated lymphocyte in spite of 24 hour exposure and intimate cell to cell contact. The control target cells, a line of human hepatoma cells, were readily destroyed. A quantitative cytotoxicity assay was devised which involved measuring the residual radioactivity of 3-H-Thymidine prelabeled target cells following exposure to activated lymphocytes. Employing this assay, we confirmed the morphologic results and showed that BeWo cells were resistant to the cytotoxic effects of lymphocytes activated by phytohemagglutinin, pokeweed, and allogeneic cells in mixed lymphocyte cultures. Moreover, BeWo were resistant to injury over a wide range of killer to target cell ratios. Significant killing of the BeWo cells occurred only after prolonged exposure (48 and 72 hours) to the activated lymphocytes. Exposing the target cells briefly to trypsin augmented the killing of control cells but not BeWo. These observations suggest that one mechanism which may assist the fetus (or a choriocarcinoma) in its immunologic survival is the intrinsic resistance of trophoblast to lymphocyte mediated cytotoxicity.

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THYMOSIN TREATMENT OF DI GEORGE SYNDROME. Ameeta K. Bamzai, Roberto R. Kretschmer, Richard M. Rothberg, Samuel P. Gotoff, Pritzker School of Medicine, University of Chicago, Michael Reese Hospital and Medical Center, Wyler Children's Hospital, Department of Pediatrics, Chicago.

A 2½-month-old infant with complete DiGeorge syndrome was treated with bovine thymosin fraction V. Initial evaluation revealed normal numbers of blood lymphocytes, 29% EAC rosettes, 41% sIg lymphocytes and no T-rosettes. T-rosettes rose to 15% upon in vitro incubation with thymosin. There was no in vitro response to PHA, PWM, SLO, candida or allogeneic cells, and delayed skin reactivity to SKSD, candida and DNCB was absent. IgG, A, M and E were 175 mg%, 15 mg%, 40 mg% and 10 Iu/ml respectively. He received 10 mg/day of thymosin for 21 days, followed by 20 mg/day for an additional 17 days, and weekly injections of the same dose thereafter. During therapy the lymphocyte count did not change, but T-rosettes rose to 26% while EAC rosettes and sIg lymphocytes decreased to 14% and 10% respectively. Lymphocytes remained unreactive in vitro to PHA, PWM, candida and allogeneic cells, but reacted to SLO (S.I.=42). Delayed skin reactions remained negative. IgG, A and M were 120 mg%, 20 mg% and 62 mg% at 4 months of age, while IgE rose to 255 Iu/ml. No specific antibodies were detected. He died of sepsis at 4½ months of age. In the postmortem no thymus or parathyroids could be found, and the lymphoid tissue revealed depleted T-dependent areas with poorly developed germinal centers. Similar partial reconstitution of T-cell functions, presumably resulting from the maturation of null-cells, have been observed in other immunodeficiencies treated with thymic extracts.

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THYMOSIN EFFECT IN PATIENTS WITH DIGEORGE SYNDROME. Douglas J. Barrett, Morton Cowan, Donna Guenther, Arthur J. Ammann, and Diane W. Wara Pediatric-Immunology University of California Medical Center, San Francsico, CA.

The effect of thymosin on T-cell function was studied in 5 patients with DiGeorge Syndrome. Initially all had low numbers of T-cell rosettes(TCR), and decreased lymphocyte responses to phytohemagglutinin (PHA) and allogeneic cells (MLC). In 3 of 4 patients tested the %TCR was significantly increased above baseline values (2 became normal) when incubated in vitro with thymosin. MLC reactivity nearly doubled with in vitro thymosin incubation in 2 patients, although the stimulation index remained abnormal. While awaiting fetal thymus transplants 4 patients received in vivo thymosin therapy. After 2 wks at 1 mg/kg/day 1 patient demonstrated normal %TCR, PHA and MLC responses. A spontaneous recovery from immunodeficiency cannot be excluded. The other 3 patients had no change in their immunocompetence, although treatment periods were short. Thus, despite in vitro enahncement of cellular immune function, a consistent in vivo response to thymosin remains to be demonstrated in patients with DiGeorge Syndrome.

Patient	%TCR	%TCR (with thymosin in vitro)	%TCR (after thymosin in vivo)	Length of therapy(days)
MS	18	64	56-90	28
TH	9	20	18	14
CT	48	65	52	8
NF	18	ND	10	14
TB	41	45	ND	-

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E-ROSETTE-FORMING CELLS (E RFC) AND Tγ CELLS (SUPPRESSOR T) PRECURSORS IN CORD BLOOD LYMPHOCYTES (CBL). RESPONSIVENESS TO THYMOSIN FRACTION V. Ricardo Bernales and Joseph A. Bellanti. Dept. Ped., Georgetown Univ. Sch. Med., Wash., D.C.

CBL "null" cells prepared by nylon column filtration and E rosette depletion constitute roughly 10% of the total Ficoll-Hypaque separated mononuclear cells. This subset, although heterogeneous, is constituted mainly of cells (80%) which possess human T lymphocyte differentiation antigens and/or receptors for the Fc portion of IgG (45% to 58%). Following incubation of CBL* with thymosin (Fraction V), the following changes were seen: E RFC increased from 9% ± 10 to 28% ± 15 ($p < .05$); Tγ increased from 58.5% ± 14 to 66.8% ± 12 ($p < .05$). Employing a double rosette assay using a chicken erythrocyte-IgG complex (CEA) developed in our laboratory, instead of sheep erythrocyte-IgG complex (sheep EA), there was an increase only in the Tγ precursors from 45% ± 7 to 88% ± 5.6 ($p < .02$), but not in E RFC nor in double rosettes. Since 5 adult peripheral blood specimens tested showed an average of 5.5% double rosettes, we doubt that steric hindrance is the cause of this observation. Rather, the Tγ precursor appears to be more avid for CEA than the mature Tγ cell, or Fc receptors induced by thymosin appear before E receptors. This subset could be a regulator of maternal-fetal immune responses and play an important role in plasma cell differentiation in the fetus.

* CBL null cells

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ABNORMAL CHEMOTAXIS AND BACTERIAL KILLING BY POLYMORPHONUCLEAR LEUKOCYTES IN A CHILD WITH RECURRENT * INFECTION. W. Douglas Biggar, Andrew C. Issekutz, (Spon. by E. W. Gelfand) Departments of Pediatrics and Immunology, Hospital for Sick Children, Toronto, Ontario, Canada.

A 9-year-old boy has had recurrent staphylococcal infections localized to the skin and clinically similar to pyoderma gangrenosum for 4 years. He has had chronic gingivitis and otitis media but never sepsis. Chemotaxis in vivo, assessed by Rebuck skin windows and by the ability to form pus was impaired. In vitro, chemotaxis by polymorphonuclear leukocytes (PMN) to serum and bacterial derived chemotactic factors was abnormal. The patient's serum generated chemotactic activity normally. In vitro, phagocytosis of bacteria by PMN was normal. However, killing of both catalase positive and catalase negative bacteria by the patient's PMN was markedly abnormal. The patient's serum did not inhibit chemotaxis or bacterial killing by control PMN. Resting and latex-stimulated oxygen consumption, hexose monophosphate pathway activity, nitroblue tetrazolium dye reduction and hydrogen peroxide production by PMN were normal. Leukocyte alkaline phosphatase, glucose-6-phosphate dehydrogenase, myeloperoxidase, lysozyme and β-glucuronidase were normal. Spontaneous release of lysozyme and β-glucuronidase from PMN was not elevated and their extracellular release increased normally during phagocytosis. This abnormality of PMN function appears to be different from those previously described and emphasizes the importance of studying such patients in order to further our understanding of PMN function.