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ISOLATION OF FOUR UNUSUAL PEDIATRIC SOLID TUMOR CELL LINES. Paul T. Peebles, Timothy Trisch, Alex G. Papageorge. Lab. of Tumor Virus Genetics, Natl. Cancer Institute, Bethesda, Md.

Four well-characterized, unique cell lines are now available for cancer, immunological, and cell differentiation research. They are derived from malignant melanoma, leiomyoblastoma, osteosarcoma, and Wilm's tumor in P-3 containment facilities in the absence of viruses and other human-animal cell lines. True Wilm's tumor and leiomyoblastoma cell lines, and a malignant melanoma line faithfully producing melanin, and a highly transformed non-fibroblastic osteosarcoma cell line have not been previously existent. All have been in culture for greater than two years and are transformed growing easily in soft agar suspension. They are human by immunofluorescence, karyotype, and G-6-PD (type B) isoenzymes. All cell lines have been studied extensively by electron microscopy. Extensive virological, immunological, and biochemical studies demonstrate little evidence for viruses similar to known RNA and DNA tumor viruses. Both early and late passages are now available from The American Type Culture Collection, Rockville, Md.

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THE MILK MONONUCLEAR PHAGOCYTE IS A MACROPHAGE. Jane Pitt, (Spon. by M. Katz), Columbia Univ. Col. Phys. & Surgs., Dept. of Pediatrics, New York.

Mononuclear phagocytic cells (MM) account for 86±2 (±1 SD) of the 1.8±.2x10^6/cc milk leukocytes in the first week of lactation. MM were isolated by differential centrifugation and glass adherence and their state of differentiation studied with histochemical immunologic, functional and metabolic assays. 31±9% of glass adherent latex ingesting cells stained for peroxidase activity compared to 100% of similarly selected blood mononuclear phagocytes (BM). 83±9% MM and 97±2 BM formed rosettes with SRBC coated with IgM anti-SRBC and the first four complement components (C3bSRBC). 96±3% MM and 97±2% BM formed rosettes with IgGSRBC. Titration of the IgGSRBC to BM or MM ratio required for rosetting revealed that MM had > 4 fold avidity for IgGSRBC than BM. MM spread and elongate on glass more rapidly than BM. This phenomenon, the low peroxidase activity, and high avidity for IgGSRBC of MM all suggest that the MM is a macrophage (MØ). IgGSRBC phagocytosis occurred in 85±4% MM and 90±5% BM under similar conditions but C3bSRBC phagocytosis occurred in neither MM nor BM suggesting that the MM is not an activated MØ. MM and BM IgGSRBC phagocytosis were completely inhibited by NaF. BM pretreated with cell free human milk (MS) resembled MM in the degree of spreading on glass, number of lysosomes, low level of peroxidase and presence of lipid droplets. Intracellular IgA, previously shown to be released from MM (Pittard et al., Ped. Res., vol. 11, No. 4, 721, 1977) was detected by immunofluorescence both in MM and in BM cultured for 24 hours in MS.

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IMMUNOREGULATION BY BREAST MILK CELLS. William B. Pittard,III,Kathleen Bill.(Spon. by Avroy Fanaroff) Dept. of Peds, CWRU, Cleveland, Ohio.

Although B cells capable of synthesizing IgG and IgM have been identified in human milk, only IgA synthesis is measured in vitro. These data suggest that milk lymphocyte differentiation is a regulated process and that there may be a specific milk cell factor capable of stimulating differentiation of IgA bearing B cells. To determine if such a factor existed we cultured lymphocyte/macrophages from early(<5 days)and late(>8 days)milk and subsequently added small aliquots of their cell free culture media to peripheral blood lymphocyte(PBL)cultures. Milk cell cultures contained 2,4 and 6x10^6 macrophages(Φ)/ml of media and a variable number of lymphocytes while the PBL cultures contained 2x10^6 lymph/ml of media. The release of IgA by PBL was quantitated using double

Mean ±S.E. PBL IgA Synthesis ng/ml

		2 x 10^6Φ		4x10^6Φ	6x10^6Φ
	Control	.025ml	.050ml .10ml	.10ml	.10ml
Early n=11	133±27	196*±49	263*±58 267±93	505±353	1450±968
Late n=6	164±43	81±30	93±27 118±45	139±25	202*±35

*p<.05 antibody radioimmunoassays. Cell free media from both early and late milk cell cultures significantly stimulated IgA synthesis. The stimulatory factor concentration was greatest in early milk cell cultures and the effect altered with increasing amounts of the regulatory factor. These data indicate that human milk cells release a regulatory factor capable of stimulating IgA synthesis by PBL and suggest a major role for colostrum in effecting active local immunity in the recipient newborn.

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DIMINISHED T CELLS FOLLOWING NEONATAL STEROID THERAPY Elena R.Reece,Tania Gunn,Kay Metrakos,Eleanor Colle. McGill Univ.,Children's Hosp.,Dept. Ped.,Montreal

Patients treated at birth with hydrocortisone for the respiratory distress syndrome(RDS) were reassessed at 5 yrs of age for growth & development,neurologic status,immunologic status & incidence of infection. The pts include 10 who received neonatal steroids,6 placebo plus 4 non-placebo RDS controls. Studies included intelligence & developmental tests,EEG's,Immunoglobulin & complement levels,diphtheria & tetanus titers,T & B cell enumeration,thymosin induction in 3 pts and delayed skin tests. The percentages of T & B cell subclasses are shown in the table.

	T	C3	Fc	SmIG
Steroid(10)	53.3 ±11.1	20.1 ±8.2	7.0 ± 4.0	11.0 ± 4.3
Control(10)	70.0 ± 7.2	13.1 ± 4.2	5.1 ± 3.5	8.7 ± 1.9
	p < 0.0025	p<0.025	NS	NS

The steroid group had a marked reduction in T cells associated in some with increased cells with C3 receptors. No significant differences were observed in numbers of cells with Fc receptors or surface immunoglobulin(SmIG) or in other immunologic parameters. There was an increased incidence of bronchopneumonia & otitis in the steroid group. Thymosin incubation led to an 11% increase in T cells in one steroid pt while no increase was seen in 2 RDS controls or adult controls. No significant differences were seen in hgt,wgt or head circumference(25-50%ile) or IQ's which were 108 ±11 for controls and 102 ±11 for steroid pts.

The data suggests that followup of infants who receive perinatal steroids should include evaluation of the immune system.

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PURINE NUCLEOSIDE PHOSPHORYLASE (PNP) DEFICIENCY WITH CELLULAR IMMUNE DEFICIENCY. K. Rich, W. Arnold, I. Fox, T. Palella. Div Immunol., Children's Mem. Hosp., Chicago, Dept. of Med U. of Illinois and U. of Michigan (Spon. L.M. Pachman)

Inherited deficiencies in purine metabolism can be associated with immunologic abnormalities. We report the 5th family deficient in PNP and cellular immunity. The proband, a 5-yr-old, had .08% of normal erythrocyte PNP. He presented at 3½-yrs with a history of mild increase in frequency of infections. He had a severe autoimmune hemolytic anemia and at 4½-yrs developed severe ataxia and tremor. He was lymphopenic (<300/mm^3) had an absolute decrease in T- and B-lymphocytes and was unresponsive to phytohemagglutinin in vitro. Response to allogeneic cells was normal. IgG and IgM were increased and a monoclonal gammopathy (IgG4λ) was present. Other antibody functions were intact (non-reactive Schick test, normal isoagglutinins, and development of influenza antibodies after infection). Erythrocyte adenosine deaminase was normal. Serum uric acid was 1.9 mg/dl and plasma and urine inosine were increased (38 μM, 13.3 mmole/gm creatinine). Urine guanosine was also increased (4.15 mmole/gm creatinine). This patient confirms the role of purine catabolism in T-lymphocyte function. Also, the unusual neurological findings, monoclonal gammopathy and hemolytic anemia suggest that clinical manifestations of PNP deficiency may be more heterogenous than previously appreciated.

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C1r DEFICIENCY: PRESENTATION AS A MILD LUPUS-LIKE SYNDROME. K. Rich, H. Gewurz. Div. of Immunol., Children's Mem. Hosp.; Dept. of Immunol., Rush Med. College, Chicago.

The congenital absence of early complement components may be associated with autoimmunity. We report the 4th patient deficient in C1r, a 14-Yr-old Puerto Rican female who presented with vague migrating arthralgia. She had a history of possible rheumatic fever and post varicella encephalitis but no unusual susceptibility to infection and no skin rash. She was normotensive and physical examination was normal. She had mild proteinuria (300 mg/24 Hr) and hematuria but no cylinduria. Creatinine clearance was normal. Renal biopsy showed mild mesangial proliferation and focal IgG and C3 basement membrane deposition. Immunoglobulins were elevated. Rheumatoid factor was present (1:640) and antibodies to Sm antigen were present (1:10^3) although antibodies to double stranded DNA were absent. High titers of immune complexes were detected by Raji cell assay. Total hemolytic complement was < 1%. Hemolytic C1 was < .1%. Other components were normal. The defect in C1 was found to be C1r by normal levels of C1q protein, 2/3 normal C1s protein, inability to correct the defect in hemolytic C1 with two C1r deficient sera, and no C1r protein. Thus, we confirm that absence of C1r may be associated with autoimmunity but the minimal clinical manifestations suggest a wider spectrum than previously appreciated.