Clin. exp. Immunol. (1980) **42,** 355–363.

T lymphocyte subpopulations and Ia-positive T cells in patients with immunodeficiency

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(Accepted for publication 30 May 1980)

SUMMARY

T lymphocyte subpopulations (Tγ and Tμ) were studied in a group of 36 adult patients with immunodeficiency. Proportions and numbers of Ia(+) T cells were also studied in comparison to 46 normal adult controls. Values for per cent and total numbers of Tγ and Tμ cells indicated no uniform abnormality. Mean normal percentage of Ia(+) T cells was 2·4% whereas 16 of 29 immunodeficient patients showed elevated proportions and absolute numbers of Ia(+) T cells. Striking fluctuation in proportions of Ia(+) T cells was noted in serial studies of five immunodeficient subjects in contrast to similar analyses of normal controls. A correlation ($P < 0{\cdot}01$) was recorded between absolute numbers of Ia(+) T cells in immune deficiency patients and numbers of Tμ cells. Depletion of Tγ cells by EA rosetting in patients with late-onset primary acquired hypogammaglobulinaemia did not result in significant change in IgG or IgM synthesis when Tγ-depleted T cells were co-cultured with normal B cells. Depletion of Ia(+) T cells likewise did not significantly influence Ig synthesis in co-culture with normal or immune-deficient B cells. These studies emphasize the complexity of defects present among any large group of patients with immune deficiency.

INTRODUCTION

Previous studies of patients with various types of immunodeficiency have provided considerable insight into the normal maturation and control of the immune system. Thus, most patients with X-linked hypogammaglobulinaemia appear to lack B cells in the peripheral blood (Cooper & Lawton, 1972; Hayward & Greaves, 1975) and are therefore incapable of generating an effective humoral immune response to various infectious agents. Patients with other forms of immunodeficiency, including that generally described as late-onset primary hypogammaglobulinaemia, probably represent a spectrum of defects of maturation or regulatory mechanisms acting at various levels within the immune system. Waldmann *et al.* (1974) and subsequently others have shown that the T cells from some patients suppress immunoglobulin production by normal B cells. However, an overactive suppressor T cell mechanism cannot account for the reduction of circulating immunoglobulins nor for the cell-mediated immunodeficiency in many of the patients with 'variable' hypogammaglobulinaemia (Broom *et al.*, 1976; De la Concha *et al.*, 1977). It is

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0099-9104/80/1100-0355$02.00

more likely that any of a variety of discrete lesions within the network of immunoregulatory control is at fault in many of these patients.

Recently two functionally distinct subpopulations of T cells have been defined by Moretta *et al.* (1977b). T cells bearing receptors for Fc of IgG (Tγ) appear to be capable of functioning as suppressor cells whereas T cells bearing Fc IgM receptors (Tμ) may act as helper cells. These two T cell subpopulations show discrete ultrastructural differences when studied by electron microscopy, and Tμ cells also show characteristic particulate cytochemical granular inclusions of neutral esterase (Grossi *et al.*, 1978). Tγ cells lack these esterase granules. This study extends the previous observations on T cell subpopulations by measuring the numbers of Tγ and Tμ cells in a large group of patients, most of whom could be classified as having late-onset primary hypogammaglobulinaemia.

Recently, it has been suggested that T cell subpopulations bearing Ia-like antigens might be involved in immune suppressor mechanisms (Albrechtsen, Solheim & Thorsby, 1977; Parish & McKenzie, 1977; Pierres *et al.*, 1978). In fact, the Tγ cell subset itself contains a significant proportion of Ia(+) cells (Greaves *et al.*, 1979). Our own preliminary data suggest that proportions of T cells bearing human Ia-like antigens are markedly increased in some patients with late-onset hypogammaglobulinaemia (Morito, Bankhurst & Williams, 1980). In contrast, several recent reports have also suggested that Ia-positive T cells might function in some instances as helper cells (Fu *et al.*, 1978; Chiorazzi, Fu & Kunkel, 1979). In this context, we have measured Ia-positive T cells in immunodeficient patients to see if there is any correlation with the numbers of Tγ and Tμ cells. We have also done experiments to test whether the removal of Tγ or Ia-positive T cells has any effect on T cell suppression of *in vitro* immunoglobulin production.

MATERIALS AND METHODS

Patients studied. Thirty-six patients with well-documented immunodeficiency were studied. Nearly all the patients had serum IgG levels of 150 mg/100 ml or less before gammaglobulin treatment; panhypogammaglobulinaemia involving IgA and IgM was also present in the majority. Four patients had X-linked agammaglobulinaemia and one of these had a chronic echovirus myositis. One infant with Di George's syndrome and one patient with selective IgA deficiency were included in the study. Thirty patients had late-onset primary hypogammaglobulinaemia; there was evidence of an additional defect in cell-mediated immunity in five of these patients. These cellular immune defects were manifested by profound depression of *in vivo* delayed-type hypersensitivity when skin-tested with a standard panel of antigens as well as a marked decrease in the *in vitro* lymphocyte response to a number of test antigens. The remaining 25 patients in this group showed panhypogammaglobulinaemia but in general had normal or borderline *in vivo* or *in vitro* cellular hypersensitivity responses. Eight of the 30 patients with late-onset hypogammaglobulinaemia were chronically ill with repeated episodes of respiratory infection. Five of these patients were receiving plasma infusions every 3 weeks in addition to their standard regime of parenteral gammaglobulin injections.

Lymphocyte cell surface marker studies. During the course of regular monthly clinic visits, peripheral blood lymphocytes were isolated using heparinized blood samples and Ficoll–Hypaque centrifugation. In all the studies of T or B cell subpopulations, mononuclear cells harvested from Ficoll–Hypaque were depleted of adherent cells by 45-min incubations at 37°C on glass petri dishes as previously described (Bankhurst & Williams, 1975). Proportions of T cells bearing receptors for Fc of IgG or IgM (Tγ and Tμ cells respectively) were determined as previously described by Moretta *et al.* (1975, 1976) after T rosetting using sheep erythrocytes treated with neuraminidase. All such neuraminidase E-rosetted mononuclear cells were studied after overnight incubation of T cells in 10% foetal calf serum–RPMI medium at 37°C in 5% CO_2–air to allow for shedding of any adsorbed or cytophilic serum proteins. Absolute numbers of Tγ and Tμ cells were based on total white blood cell counts and differentials performed on the same samples.

The presence of human Ia-like antigen on T cells was determined on neuraminidase E-rosetted cells after a 12–14-hr incubation at 37°C in 10% FCS–RPMI and 5% CO_2–air. T cells

were incubated with rabbit or chicken anti-human Ia antiserum, washed and stained with fluorescein–conjugated goat anti-chicken or anti-rabbit gammaglobulin. A total of 200–300 cells were examined using incident light and a Zeiss phase-immunofluorescence microscope.

The rabbit or chicken anti-Ia reagents were prepared by the method described by Welsh & Turner (1976) (see also Greaves *et al.*, 1979) in which immune precipitates formed in 1% agarose gels between rabbit anti-Ia and cell membrane lysates of Ia-positive cultured B cell lines were injected subcutaneously into rabbits or chickens. An equal volume of incomplete Freund's adjuvant was emulsified with the agarose-embedded immune precipitates and three subcutaneous injections were given at 10-day intervals followed by bleeding 7–10 days after the third injection. The chicken anti-Ia antiserum was then absorbed with insolubilized rabbit IgG, and twice with ⅓ volume of fresh washed human thymocytes obtained from children during the course of cardiac surgery. Absorption was carried out for 4 hr at 4°C followed by centrifugation at 3,500 *g* and ultimately clearing by ultracentrifugation at 30,000 *g*. These anti-Ia reagents showed no positive immunofluorescence with human thymocytes, mature granulocytes, platelets, or human red blood cells. However, strong membrane staining was noted with B cells from patients with chronic lymphocytic leukaemia, various Ia-positive human B cell lines, and Ia-positive cells from patients with non-T acute lymphoblastic leukaemia (Greaves *et al.*, 1975, 1977). Avian antibody was found not to react in its whole state with Fc receptors on human mononuclear cells, thus avoiding inadvertent reactions with the so-called 'third population' of lymphocytes possessing Fc receptors (Winchester *et al.*, 1975; Horwitz & Lobo, 1975). In addition, the fluorescein-labelled goat anti-chicken reagent also avoided reactivity with Fc receptors (Alexander & Sanders, 1977).

Functional studies. In several instances an attempt was made to relate cell surface marker profiles with immunological function *in vitro*. An assay was employed which utilized normal human peripheral blood B cells stimulated *in vitro* with pokeweed mitogen and co-cultured with allogeneic normal lymphocytes or mononuclear cells from various patients with immunodeficiency with subsequent measurement of supernatant IgM and IgG by radioimmunoassay (Morito, Bankhurst & Williams, 1979). Details of this technique have been described previously (Morito *et al.*, 1980). Briefly, after Ficoll–Hypaque gradient separation, cells were washed three times with Hanks' balanced salt solution. T lymphocytes were prepared by E neuraminidase-treated erythrocytes; rosetting cells were separated from those non-rosetting by centrifugation of E neuraminidase rosetting cells through a Ficoll–Hypaque gradient. The non-rosetting cells left at the Ficoll–Hypaque interface were considered to represent the 'B' cell fraction plus null cells. Cells forming E neuraminidase rosettes were found to consist of 95% E rosetting cells on reassay after separation through Ficoll–Hypaque gradients. Cell viability in all preparations used in lymphocyte co-culture experiments was >95% by trypan blue dye exclusion.

In mixture experiments between normal control and immune deficiency patients' lymphocytes, equal numbers of cells were mixed to give a final total cell concentration of $3{\cdot}0 \times 10^5$ cells in a final microplate well volume of 250 μl. In such experiments, B lymphocyte preparations were adjusted to a concentration of 6×10^4 cells per microplate well and to this constant number of B lymphocytes, varying ratios of T cells were added. After 7 days of co-culture in the presence of a 1 : 200 dilution of pokeweed mitogen, microtitre plates were centrifuged (500 *g* for 10 min) and radioimmunoassay completed for supernatant content of IgG and IgM (Morito *et al.*, 1980). Tγ cell preparations were performed as originally described by Moretta *et al.* (1976). After 7 days of culture, cell viability again as monitored by trypan blue dye exclusion was generally greater than 85–90%. T cells from normal controls as well as from patients with immunodeficiency (where specific determinations of Tγ, Tμ and Ia-positive T cells had been performed) were added to the *in vitro* system to provide help or suppression. This *in vitro* assay system was similar to that initially employed by Waldmann *et al.* (1974) except that a wide range of T cell concentrations ($0{\cdot}6$ to $2{\cdot}4 \times 10^5$) was added to 6×10^4 B cell fraction. In some instances, treatment of isolated T cells with whole rabbit anti-Ia antiserum and fresh rabbit serum as a source of complement was utilized to evaluate the relative contribution of Ia-positive T cells to cellular interaction. Normal rabbit serum was used as a control in these circumstances.

Normal controls. Forty-six normal adults, aged 20–50, of both sexes served as controls.

Table 1. T cell markers in groups of immune deficiency patients studied

	Per cent T	Total T	Per cent Tγ	No Tγ	Per cent Tμ	No Tμ	Per cent Ia(+) (T)	No Ia(+) (T)
X-linked agammaglobulinaemia								
J.W.	69	2,816	4	113	21	591	—	—
A.W.	62	1,637	3	49	9	147	—	—
R.	84	3,984	16	637	17	677	38	1,514
S.G.	83	2,005	12	240	7	140	4	80
Common variable immunodeficiency								
Severe cell-mediated immunity impairment								
S.M.	82	5,614	34	1,909	70	3,930	14	786
C.C.	41	575	9	52	22	127	—	—
G.R.	81	632	20	126	27	170	4	25
A.W.	86	8,013	3	240	12	961	17	1,362
J.B.	89	2,990	16	478	39	1,166	50	1,495
Mean		3,665	16·4±11·8	561±770	34±22	1,270·8±1,557	21·3±20	917±670
Borderline or normal cell-mediated immunity								
W.	73	4,774	10	477	21	1,003	—	—
J.F.	66	1,324	15	199	52	688	23	304
E.O.	84	1,199	21	252	72	863	21	252
J.W.	88	2,265	20	453	48	1,087	4	91
S.C.	76	675	8	54	40	270	12	81
C.C.	82	1,100	14	154	41	451	4	44
J.H.	76	1,956	13	254	49	958	4	78
P.E.	80	1,973	12	236	33	651	4	79
F.L.	74	5,028	13	654	57	2,866	5	251
B.L.	88	1,762	14	247	58	1,022	n.d.	57
C.M.	81	1,424	8	115	47	812	4	57
B.R.	80	2,001	16	320	64	1,280	7	140
J.B.	82	2,300	11	253	43	989	16	368
J.C.	84	771	6	46	50	386	12	93
K.M.	71	2,901	11	319	22	638	12	348
R.S.	87	1,890	7	132	50	945	18	340
W.F.	82	732	14	103	55	403	15	110
E.F.	83	1,262	13	164	57	719	18	227
M.B.	74	2,812	7	197	20	562	6	169
B.P.	78	1,372	8	109	12	164	8	110
B.K.	82	2,479	16	397	48	1,190	46	1,140
W.B.	64	923	3	27	45	415	35	323
W.S.	73	2,593	11	285	53	1,374	25	648
J.S.	86	2,504	16	400	60	1,502	32	801
H.C.	79	1,422	14	341	19	270	3	43
Mean		1,977·7±680	12·4±4·9	247·5±151	45·0±15	860±552	14·5±12	256±267
Di George's Syndrome								
J.R.	49	3,748	8	301	18	675	26	974
Selective IgA deficiency								
C.	62	1,272	9	114	53	674	1	13
Normal controls (46)								
Mean ±s.d.	70·8	1,882±718	9·1±4·7	173±108	48±9·0	908±387	3·5±1·9	59±60·2

RESULTS

Cell surface markers

Normal values for Tγ, Tμ and Ia-positive T cells determined among control subjects are shown in Table 1. Mean per cent and total numbers of Tγ and Tμ cells showed some variation with normal mean Tγ cell proportions of 9·1±4·7% and 173±108/mm³ respectively. Mean normal per cent and total numbers of Tμ cells were 48·0±9·0 and 908±387/mm³. The mean normal percentage of Ia-positive T cells was 2·4%. Values for per cent and total numbers of Tγ and Tμ cells in the group of immunodeficiency patients studied are also shown in Table 1 and appear to indicate no uniform abnormality. The proportions of Ia-positive T cells in the immunodeficiency group ranged from 4 to 50% (Table 1).

Sixteen of the 29 immunodeficient patients had elevated proportions and total numbers of Ia-positive T cells. Some degree of correlation appeared to exist between proportions or numbers of T cells bearing Ia antigen and total number of Tμ cells/mm³ (Fig. 1). In addition, serial studies of Ia-positive T cells were performed in five adult patients with late-onset hypogammaglobulinaemia. When compared to normal controls, these patients showed a remarkable variation in proportions of Ia(+) T cells (Table 2). No correlation could be noted among percentages of Ia(+) T cells and clinical status in individual patients serially studied.

Functional studies

If Tγ cell or putative suppressor T cell overactivity were directly related to hypogammaglobulinaemia, it is possible that elimination of Tγ cells might be associated with diminution of demonstrable *in vitro* suppression as measured by co-culture with normal peripheral blood lymphocytes in the presence of pokeweed mitogen. Accordingly, lymphocytes from several hypogammaglobulinaemic patients with additional cellular immune defects were studied before and after elimination of Tγ cells by EA rosetting using ox erythrocytes sensitized with optimal dilutions of IgG rabbit anti-ox erythrocyte antibody. Elimination of Tγ cells from both normal subjects and six patients resulted in no significant increase in IgG or IgM by co-cultures of lymphocytes from patients and normals stimulated with pokeweed mitogen. Representative

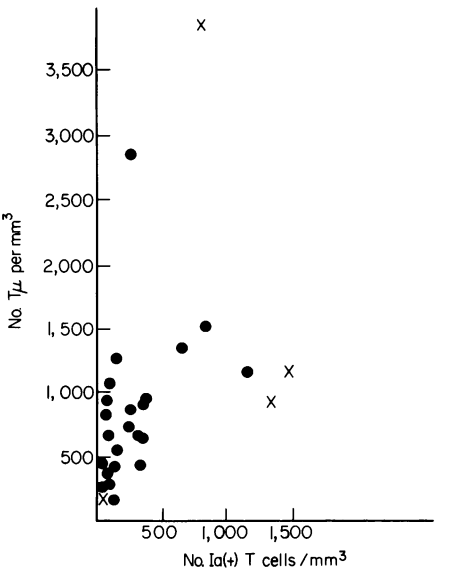

Fig. 1. Relationship of absolute numbers of Ia(+) T cells in immune deficiency patients and numbers of Tμ cells. (●) Common variable and (×) X-linked. Calculation of Spearman rank correlation coefficient showed an r_s value of 0·5694 (P <0·01); similar calculations for rank correlation coefficients in 25 normals (data not shown) gave an r value of 0·4548 (P <0·05).

Table 2. Variation in per cent Ia(+) T cells among individual patients with combined immune deficiency studied serially

Subject		Per cent T rosettes	Per cent Ia(+) T cells	Per cent SmIg
Patients				
1. J.B.	1/8/78	89	7	4
	30/8/78	80	50	6
	5/1/79	90	17	8
	6/4/79	65	5	6
	26/4/79	86	3	2
2. B.K.	2/8/78	82	4	5
	28/9/78	75	46	8
3. V.S.	9/8/78	73	25	4
	28/9/78	69	20	3
	25/4/79	83	5	7
4. W.B.	9/8/78	64	35	3
	2/9/78	68	15	4
	16/4/79	61	4	16
5. J.S.	3/10/78	86	23	1
	10/10/78	62	32	0
	23/1/79	79	6	2
	9/4/79	81	1	1
Normals				
1.	30/8/78		4	9
	1/10/78		3	8
	2/4/79		2	10
	1/5/79		1	7
2.	1/8/78		3	12
	15/10/78		2	8
	15/11/78		3	11
	1/4/79		4	7
3.	15/8/78		2	8
	1/9/78		3	8
	30/11/78		1	10
	1/2/79		0	9
4.	18/8/78		2	11
	13/9/78		3	12
	14/10/78		1	8
	1/2/79		0	10
	4/4/79		2	9

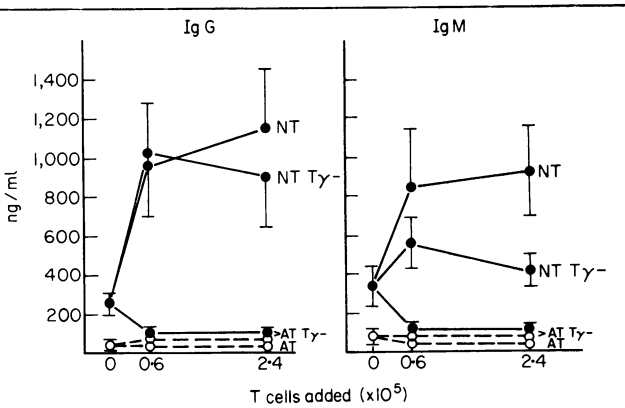

Fig. 2. Results of depletion of Tγ cells by EA rosetting on IgG and IgM synthesis from normal T cells (NT) or late-onset primary acquired hypogammaglobulinaemic T cells (AT) co-cultured with normal B cells (●——●) or adult acquired agamma B cells (○——○). Points indicate mean ±s.e. of six patients with late-onset primary adult acquired agammaglobulinaemia as well as an equal number of controls.

Table 3. Results of depletion of Ia(+) T cells on Ig synthesis by B cells and co-cultured T cells from six normal subjects and four patients with late-onset primary hypogammaglobulinaemia

	Normal B cells*		Agamma B cells*	
	+normal T	+agamma T	+normal T	+agamma T
IgG produced (μg)				
Ia(−)†	1,396·9±289·7‡	90·0±56·3	40·2±19·8	51·1±39·9
Control	1,732·6+455·6§	108·4+54·8	37·6+18·2§	31·5+17·9
IgM produced (μg)				
Ia(−)	888·0±244·6	78·8±35·2	55·6±25·8	99·2±49·4
Control	1,037·9+316·3	114·0+58·1	41·3+21·1	76·4+39·9

* Anti-Ia treated or non-treated T cells (6×10^4) were mixed with 6×10^4 B cells per microplate well.

† Ia(−) refers to T cells depleted of Ia(+) T cells; control refers to T cells treated with normal rabbit serum.

‡ All data are shown as ng/ml (mean ± s.e.) of Ig production in triplicate experiments.

§ Normal B cells cultured alone without any T cells produced 350 ± 207 ng/ml of IgG and 208±50·7 IgM; agamma B cells cultured alone without any T cells generated 20·2±15·1 ng/ml IgG and 30·5±15 IgM.

results of such Tγ elimination experiments in normal subjects and a group of six patients with late-onset hypogammaglobilinaemia are shown in Fig. 2.

Similar studies were done to ascertain whether Ia(+) T cells in both normal subjects and hypogammaglobulinaemic patients played any part in regulating *in vitro* immunoglobulin production. When Ia(+) T cells were eliminated from neuraminidase rosette-separated T cells by treatment with rabbit anti-Ia antiserum and complement, a slight decrease in both IgG and IgM production was noted in lymphocyte co-cultures from normal subjects. Analysis of the effectiveness of elimination of Ia(+) T cells by anti-Ia antiserum and complement was confirmed by analysis of Ia(+) T cells before and after treatment. Representative data illustrating these experiments are shown in Table 3. A slight increase in Ig production was noted in the co-cultures from patients with late-onset hypogammaglobulinaemia. However, these changes did not reach statistical significance by χ^2 analysis.

DISCUSSION

The profile of peripheral blood T cell subsets documented among the immunodeficiency patients studied here does not show any uniform pattern. Numbers of Tγ cells ranged from 49–637/mm^3 and Tμ cells from 140–677/mm^3 in the four X-linked agammaglobulinaemic patients (Nos 1–4, Table 1). Similar wide ranges were recorded among the patients with late-onset hypogammaglobulinaemia with or without concurrent impairment of cell-mediated immunity. Thus, some patients with primary hypogammaglobulinaemia showed high proportions or total numbers of Tγ cells whereas in others the values were considerably lower than normal. The same was true of Tμ percentages and total numbers. No uniform pattern of Tγ or Tμ profile was apparent or could be correlated with clinical picture in individual subjects. An interesting apparent correlation between absolute numbers of Tμ cells and Ia(+) T cells was noted in patients with late-onset hypogammaglobulinaemia. Double-labelling cell surface marker analysis in a small number (five) of these individuals indicated that an average of 35% of Tγ cells were Ia(+). No Ia(+) T cells were detected in the non-Tγ T cell subpopulations.

Our data extend and confirm the broad ranges of both proportions and absolute numbers of Tγ and Tμ cells in immunodeficient patients previously recorded in two different studies (Moretta *et al.*, 1977a; Gupta & Good, 1978). Moreover, the suppression of Ig synthesis by patient T cells co-cultured with normal B lymphocytes was not directly attributable to Tγ cells, since virtually complete depletion of Tγ cells did not reverse this inhibition. Furthermore, in

addition to the four patients (J.B., B.K., J.S., H.C., Table 1) with potent suppressor activity shown in Table 3, three other patients (E.O., A.W., J.C.) with late-onset hypogammaglobulinaemia have consistently shown T cell suppression of Ig synthesis by normal B cells although these tests were not done in parallel with the T cell subset assays described here (T. Platts-Mills, personal communication). Only one of these patients had a relatively high percentage of circulating Tγ cells.

We were also unable to equate suppressor T cell activity with Ia(+) T cells. Thus, elimination of Ia(+) T cells from the T cells of patients with known suppressor activity had no effect on suppression of Ig production by either normal or patient B cells. The apparent statistical correlation between numbers of Ia(+) T cells and Tμ cells might suggest that Ia(+) T cells, known to be a subpopulation of Tγ cells (Greaves *et al.*, 1979), may be related to help rather than suppression. Several reports supporting this thesis have recently appeared (Fu *et al.*, 1978; Chiorazzi *et al.*, 1979) but more direct analysis of this possibility is required.

This study suggests that neither Tμ, Tγ or Ia(+) T cells are directly involved in suppression of *in vitro* immunoglobulin production. The study also extends the range of T cell abnormalities found in patients with late-onset hypogammaglobulinaemia. For instance, it is interesting that many of the patients studied here have previously been shown to have very low percentages of esterase-positive T cells (Matamoros *et al.*, 1979). Such patients might be expected to have very low percentages of Tμ cells although this study suggests that this is not the case. It is more likely that the T cells of these patients are not fully differentiated and that the low activity of T cell ecto-5′ nucleotidase previously described (Rowe *et al.*, 1980) and the expression of Ia antigens reflect an 'immature' cell type.

Supported in part by grants AMAI 13824-10 and AMI 36900-09 from the National Institutes of Health and in part by grants from the Kroc Foundation, the Josiah Macy Foundation and the American Cancer Society.

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