

Ecto-5'-Nucleotidase Activity in Human T Cell Subsets. DECREASED NUMBERS OF ECTO-5'-NUCLEOTIDASE POSITIVE CELLS FROM BOTH OKT4⁺ AND OKT8⁺ CELLS IN PATIENTS WITH HYPOGAMMAGLOBULINEMIA

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Research Article

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Two of four patients with congenital agammaglobulinemia and four of seven patients with common variable immunodeficiency had decreased proportions of OKT4⁺ T cells with corresponding increases in the proportions of OKT8⁺ T cells (OKT4/OKT8 = 0.60 to 1.0 as compared with 1.7±0.2 for control subjects). All four patients with congenital agammaglobulinemia, and three of seven patients with common variable immunodeficiency also had low T cell ecto-5'-nucleotidase activity (<5.5 nmol/h per 10⁶ cells). Ecto-5'-nucleotidase activity in OKT4-enriched populations isolated from four patients with low total T cell activity was 2.85±0.90 nmol/h per 10⁶ cells with 10±4% positive by histochemical stain; that in OKT8-enriched populations was 6.82±1.7 nmol/h per 10⁶ cells with 7.5±3% positive by histochemical stain. Thus, the number of ecto-5'-nucleotidase [...]

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DECREASED NUMBERS OF ECTO-5'-NUCLEOTIDASE POSITIVE CELLS FROM BOTH OKT4⁺ AND OKT8⁺ CELLS IN PATIENTS WITH HYPOGAMMAGLOBULINEMIA

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ABSTRACT T lymphocytes from control subjects were separated into subsets using monoclonal antibodies of the OKT series and complement lysis and analyzed for ecto-5'-nucleotidase activity both by quantitative radiochemical assay and a histochemical stain. T cells from 15 control subjects contained 54±4% OKT4⁺ (helper/inducer) cells and 32±3% OKT8⁺ (cytotoxic/suppressor) cells. Total T cell ecto-5'-nucleotidase activity was 10.9±2.1 nmol/h per 10⁶ cells with 25±7% positive by histochemical stain. Ecto-5'-nucleotidase activity in OKT4-enriched populations was 5.43±1.8 nmol/h per 10⁶ cells with 14±2% positive by histochemical stain; that in OKT8-enriched populations was 17.1±5.9 nmol/h per 10⁶ cells with 35±8% positive by histochemical stain.

Two of four patients with congenital agammaglobulinemia and four of seven patients with common variable immunodeficiency had decreased proportions of OKT4⁺ T cells with corresponding increases in the proportions of OKT8⁺ T cells (OKT4/OKT8 = 0.60 to 1.0 as compared with 1.7±0.2 for control subjects). All

four patients with congenital agammaglobulinemia, and three of seven patients with common variable immunodeficiency also had low T cell ecto-5'-nucleotidase activity (<5.5 nmol/h per 10⁶ cells). Ecto-5'-nucleotidase activity in OKT4- enriched populations isolated from four patients with low total T cell activity was 2.85±0.90 nmol/h per 10⁶ cells with 10±4% positive by histochemical stain; that in OKT8-enriched populations was 6.82±1.7 nmol/h per 10⁶ cells with 7.5±3% positive by histochemical stain. Thus, the number of ecto-5'-nucleotidase positive cells is decreased, especially in the OKT8⁺ subpopulation, and the low total T cell ecto-5'-nucleotidase activity seen in these patients is due to fewer positive cells rather than to substantially less activity per cell.

Our data indicate that ecto-5'-nucleotidase activity defines two subpopulations of T lymphocytes (ecto-5'-nucleotidase positive and negative), the proportions of which are markedly altered in many patients with hypogammaglobulinemia. In preliminary studies with seven patients, increased numbers of ecto-5'-nucleotidase negative T cells appeared to correlate with increased suppressor T cell activity toward in vitro immunoglobulin synthesis. Therefore, ecto-5'-nucleotidase may be a useful cell surface marker in the study of imbalances of regulatory T cell subsets in patients with antibody synthesis disorders.

INTRODUCTION

Decreased activity of specific enzymes in the purine catabolic pathway leads to profound human immunodeficiency diseases. Deficiency of lymphocyte aden-

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osine deaminase (ADA)¹ activity causes severe combined immunodeficiency (1); deficiency of purine nucleoside phosphorylase (PNP) activity leads to an isolated T cell defect (2). Both enzyme deficiencies are the results of mutations in the structural genes coding for the missing enzymes, leading to losses of activity in all tissues (3–7). In both cases, the patients' loss of immune function can be directly attributed to alterations in purine ribo- and/or deoxyribonucleoside metabolism (8–15). A deficiency of a third enzyme in the purine catabolic pathway, ecto-5'-nucleotidase (ecto-5'-NT), which is located on the external surface of the plasma membrane, has been reported in the lymphocytes of all patients with congenital agammaglobulinemia (CAG) (16, 17) and the majority of patients with common variable immunodeficiency (CVI, or adult onset hypogammaglobulinemia) (18). Unlike deficiencies of ADA and PNP, the molecular and/or cellular basis of ecto-5'-NT deficiency is not well understood. Ecto-5'-NT deficiency is restricted to lymphocytes, does not appear to be the consequence of a structural gene mutation, and causes no detectable aberrations in purine metabolism (19).

Because human peripheral blood lymphocytes are not homogeneous with respect to ecto-5'-NT activity (17, 20, 21), the deficiency in patients with CAG and CVI can be understood only by studying purified lymphocyte subpopulations. Ecto-5'-NT deficiency in CAG and CVI may be partially explained by an absence of mature B lymphocytes, which normally have three- to fivefold higher ecto-5'-NT activity than total T cell preparations (17, 20, 22). However, the majority of patients with CAG (17, 23) and CVI (24) also have decreased (one-third to one-half of normal) ecto-5'-NT activity in total T cell preparations. We have previously shown (21) that T γ cells (T cells with Fc receptors for IgG) have only one-third the ecto-5'-NT activity of T μ cells (T cells with Fc receptor for IgM); however, little is known regarding ecto-5'-NT activity in T cell subsets defined by the monoclonal antibodies OKT4 (helper/inducer) and OKT8 (cytotoxic/suppressor) (25). The experiments described here were designed to determine: (a) whether OKT4⁺ and OKT8⁺ T cells isolated from normal individuals have different levels of ecto-5'-NT activity, and (b) whether the low T cell ecto-5'-NT activity in CAG and CVI patients could be explained by altered proportions of regulatory T cell subsets. In addition, we have performed preliminary experiments to determine whether low T cell ecto-5'-NT activity in patients with CAG and CVI

can be correlated to a specific defect in T cell regulation of immunoglobulin biosynthesis.

METHODS

Patient populations. The diagnosis of CAG was based upon early onset of infections, absence of B lymphocytes, and agammaglobulinemia. For patients 2 and 3, there was also evidence for X-linked inheritance. Patient 2 is the maternal uncle of patient 3 and also has a brother with CAG; this pedigree has been previously published (26). The diagnosis of CVI was based upon increased incidence of infections beginning in late adolescence or early adulthood and depressed concentrations of serum immunoglobulins. Control subjects were healthy laboratory volunteers between the ages of 24 and 33 yr. We have previously shown that lymphocyte ecto-5'-NT activity remains constant from early childhood until the age of 40 yr (21).

Lymphocyte isolation and staining with monoclonal antibodies. Peripheral blood lymphocytes were isolated from freshly drawn heparinized venous blood by dextran sedimentation, carbonyl iron treatment to remove monocytes, and Ficoll-Hypaque density gradient centrifugation (27). Carbonyl iron treatment did not lead to selective loss of lymphocyte subpopulations as determined by T and B cell numbers, percentages of OKT4⁺ and OKT8⁺ T cells, and ecto-5'-NT activity. T lymphocytes were isolated by one cycle of rosetting with neuraminidase-treated sheep erythrocytes for 15 min at 37°C and 1 h at 4°C followed by Ficoll-Hypaque density gradient centrifugation (28). Sheep erythrocytes were lysed with 0.83% NH₄Cl. Lymphocytes isolated by this method were 93–97% T cells as determined by a second rosetting with neuraminidase-treated sheep erythrocytes or by the percentage of cells staining with monoclonal antibody Lyt3, clone 9.6 (New England Nuclear, Boston, MA) (29). For immunofluorescent staining 10⁶ lymphocytes were incubated with the specific monoclonal antibody (or control myeloma protein of the same immunoglobulin subclass) followed by F1-F(ab')₂ goat anti-mouse Ig (N. L. Cappell Laboratories, Cochranville, PA) as previously described (30). The percentage of cells showing specific staining was determined by a FACS IV cytofluorograph (Becton-Dickinson Co., Mountain View, CA) (31).

Isolation of T cell subsets. 10 million lymphocytes in 200 μ l of RPMI 1640 + 2.5% fetal bovine serum (FBS) were incubated with 200 μ l of an appropriate dilution of monoclonal antibody in phosphate-buffered saline (PBS) + 0.01 M sodium azide for 1 h at room temperature. Rabbit serum, 80 μ l, was added as a source of complement (C') and the cells were incubated at 37°C for 90 min. Under these conditions, no capping, shedding, or endocytosis of antigen-antibody complexes was observed to take place. The cell suspension was then diluted and subjected to Ficoll-Hypaque density gradient centrifugation in order to remove dead cells and cell debris. Greater than 95% of the cells recovered at the interface were viable as judged by their ability to exclude trypan blue. T cells were incubated with the monoclonal antibodies OKT8 and OKM1 (which react with T γ cells [30, 32, 33]) to enrich for OKT4⁺ cells and with OKT4 and OKM1 to enrich for OKT8⁺ cells. T cells incubated with the monoclonal antibody OKT6 (which reacts with thymocytes, but not peripheral T cells [34]) followed by C' served as the control. All isolated T cell subsets were stained with the appropriate monoclonal antibodies and analyzed with the FACS IV to determine the purity of the T cell subsets. For this purpose, cells were first stained with F1-F(ab')₂ goat anti-

¹ Abbreviations used in this paper: ADA, adenosine deaminase; CAG, congenital agammaglobulinemia; CVI, common variable immunodeficiency; ecto-5'-NT, ecto-5'-nucleotidase; FBS, fetal bovine serum; PBM, peripheral blood mononuclear cells; PWM, pokeweed mitogen.

mouse Ig to determine the percentage of cells stained with monoclonal antibodies, but not lysed by C'. In the OKT4-enriched preparation, for example, this procedure would detect residual OKT8⁺ and OKM1⁺ T lymphocytes. A second preparation of cells was then stained with the monoclonal antibody that defines the subset for which they were enriched, i.e. OKT4, in the case of OKT4-enriched cells. The percentage of cells staining with F1-F(ab')₂ goat anti-mouse Ig alone was subtracted from the percentage of cells staining with OKT4 in order to obtain the true percentage of OKT4⁺ cells in the population. OKT4⁺-enriched preparations contained 75–95% OKT4⁺ cells; OKT8⁺-enriched preparations contained 70–85% OKT8⁺ cells.

T cells with Fc receptors for IgG. The percentage of T cells bearing Fc receptors for IgG (T_γ cells) was determined by rosetting with fresh ox erythrocytes coated with IgG anti-ox (Cappel Laboratories) as previously described (35).

Enzyme assays. ADA, PNP, and ecto-5'-NT (with [¹⁴C]IMP as substrate) assays were performed as previously described (17, 36).

Histochemical stain for 5'-NT. The percentage of lymphocytes with 5'-nucleotidase activity was determined by a minor modification of the method described by Silber et al. (37). Approximately 5 × 10⁴ cells were applied to a microscope slide using a cyto centrifuge. The cells were then incubated with 5'-AMP, β-glycerophosphate, sodium potassium tartrate, and Pb (NO₃)₂ at 37°C for 18 h, fixed with 0.4% glutaraldehyde, and the stain developed by incubation in 2% (NH₄)₂S. Nonstaining cells were visualized by counterstaining for 2 min in 0.05% toluidine blue. Only those cells completely covered by a black precipitate of PbS were considered positive for ecto-5'-NT activity. Due to the intensity of the stain, we could have detected one-third to one-fourth of the activity found, but there did not appear to be cells that stained with intermediate levels of activity. A minimum of 400 cells were counted to determine the percent positive cells. The percentage of cells staining with 2',3'-AMP (<2%) served as a negative control and was subtracted from the percentage staining with 5'-AMP.

Statistics. Data were analyzed statistically with a Student's *t* test. All data are reported as mean ± 1 SD.

In vitro immunoglobulin biosynthesis. Peripheral blood mononuclear cells (PBM) were isolated from freshly drawn heparinized venous blood by Ficoll-Hypaque density gradient centrifugation (38). PBM preparations were then rosetted with neuraminidase-treated sheep erythrocytes to yield rosetting (T) cells and nonrosetting cells. Although the nonrosetting cells contained monocytes and third population cells as well as B cells, they will be referred to as "B cells" for the purpose of this discussion. 1 million cells (PBM or T cells + B cells) were cultured in 1 ml of RPMI 1640 + 10% FBS + 2 mM glutamine + streptomycin (100 μg/ml), penicillin (100 U/ml) and amphotericin B (2.5 μg/ml) at 37°C in an humidified atmosphere of 5% CO₂ in air for 7 d in the presence of 10 μl of pokeweed mitogen (PWM) (Grand Island Biological Co., Grand Island, NY, ~5 mg/ml stock solution). At the end of the culture period, the cells were spun down and the quantities of IgM and IgG secreted into the supernatant tissue culture medium were determined by radioimmunosorbent tests (39).

RESULTS

Characterization of total T cells from control subjects and CAG and CVI patients. Total T lymphocytes from control subjects and patients with CAG and

CVI were characterized with respect to ecto-5'-NT activity and the percentages of cells bearing Fc receptors for IgG (T_γ cells) and the T4 and T8 antigens (Table I). All four CAG patients studied had <55% of normal ecto-5'-NT activity in their total T lymphocytes. There was a great variation in ecto-5'-NT activity in total T lymphocytes isolated from patients with CVI, with individual values from 25 to 100% of normal. There were no obvious correlations between the degree of enzyme deficiency and age of onset or severity of disease with the CVI patients. Total T lymphocytes from control subjects contained 8.8 ± 3% T_γ cells, 54 ± 4% OKT4⁺ cells, and 32 ± 3% OKT8⁺ cells, with a ratio of OKT4⁺/OKT8⁺ cells of 1.7 ± 0.2. All but two of the patients studied had decreased percentages of T_γ cells. Two of the four patients with CAG and four of the seven patients with CVI had increased percentages of cells recognized by the monoclonal antibody OKT8, which yielded OKT4/OKT8 ratios of 0.60 to 1.0.

Ecto-5'-nucleotidase activity in OKT4-enriched and OKT8-enriched lymphocyte subpopulations. The ecto-5'-NT activity in OKT4-enriched and OKT8-enriched T lymphocyte subpopulations from control subjects and CAG and CVI patients by both quantitative

TABLE I
Characterization of T Cells from Control Subjects
and Patients with CAG and CVI

Subject	Ecto-5'-NT activity*	T _γ †	OKT4 ⁺	OKT8 ⁺	OKT4/OKT8 ratio
% total T cells					
Controls	10.1 ± 3.5	8.8 ± 3	54 ± 4	32 ± 3	1.7 ± 0.2
Mean ± 1 SD (n = 10)		(n = 15)	(n = 15)	(n = 15)	
CAG Patients					
1	5.26	8.3	41	53	0.77
2	5.42	2.7	54	38	1.4
3	3.78	ND	56	34	1.6
4	5.27	ND	45	52	0.87
CVI Patients					
5	7.90	0.2	31	49	0.63
6	4.29	4.0	45	44	1.0
7	3.27	5.3	31	52	0.60
8	3.48	12	48	35	1.4
9	10.2	0.3	53	36	1.5
10	8.30	ND	47	33	1.4
11	9.64	ND	43	43	1.0

* Nanomoles per hour/10⁶ cells.

† Percentage of T cells forming rosettes with ox erythrocytes coated with rabbit IgG anti-ox.

radiochemical assay and histochemical stain is shown in Table II. The manipulations required in staining the cells with monoclonal antibodies, incubating with rabbit serum as a source of C', and purifying the viable cells by Ficoll-Hypaque density gradient centrifugation did not significantly alter the ecto-5'-NT activity, as was found by comparing the enzyme activity in cells treated with OKT6 + C' with that in untreated T cells (data not shown). Staining T cells with OKT4, OKT8, or OKM1 also did not alter the ecto-5'-NT activity (data not shown). In seven separate experiments with six control donors, the mean ecto-5'-NT activity in OKT4-enriched T cells was 5.43 ± 1.8 nmol/h per 10^6 cells with $14 \pm 2\%$ positive by histochemical stain, that in OKT8-enriched T cells was 17.1 ± 5.9 nmol/h per 10^6 cells with $35 \pm 8\%$ positive cells by histochemical stain ($P < 0.001$). To insure that the low ecto-5'-NT activity in OKT4-enriched populations is not due to small percentages of unlysed OKT8⁺ cells, in a single experiment, peripheral blood mononuclear cells were stained with OKT4, and OKT4⁺ cells were prepared by positive selection using the FACS IV for cell sorting. In a population of 96% OKT4⁺ cells, ecto-5'-NT activity was 6.27 nmol/h per 10^6 cells with 5.3% ecto-5'-NT⁺ by histochemical stain. Since only 35% of the OKT4⁺ cells in this preparation would be expected to be ecto-5'-NT⁺, it is unlikely that all the ecto-5'-NT activity in OKT4-enriched preparations can be due to contaminating OKT4⁺ cells. Thus, populations enriched for OKT8⁺ cells had threefold more ecto-5'-NT

activity and a 2.5-fold greater percentage of ecto-5'-NT positive cells than those enriched for OKT4⁺ cells. The amount of ecto-5'-NT activity per positive cell was approximately the same (within the limitations of these methods) in the OKT4- and OKT8-enriched subpopulations.

Ecto-5'-NT activity was also measured in OKT4-enriched and OKT8-enriched lymphocytes isolated from two patients with CAG and two patients with CVI who had low T cell ecto-5'-NT activity. Three of the four patients also had increased numbers of OKT8⁺ T cells. Ecto-5'-NT activity in the OKT4-enriched populations was 2.85 ± 0.90 nmol/h per 10^6 cells with $10 \pm 4\%$ positive by histochemical stain; that in the OKT8-enriched population was 6.82 ± 1.7 nmol/h per 10^6 cells with $7.5 \pm 3\%$ positive by histochemical stain. Thus, these patients have reduced numbers of ecto-5'-NT positive T cells, especially in the OKT8⁺ subpopulation, and their low total T cell enzyme activity is due to fewer positive cells rather than to substantially less activity per cell.

Adenosine deaminase and purine nucleoside phosphorylase activities in OKT4-enriched and OKT8-enriched lymphocyte subpopulations. In order to determine whether T cell subsets differ in other enzymes in the purine catabolic pathway, adenosine deaminase and purine nucleoside phosphorylase activities were measured in OKT4- and OKT8-enriched lymphocyte populations isolated from two healthy donors. There was no significant difference in either enzyme activity

TABLE II
Ecto-5'-Nucleotidase Activity in Purified T Cell Subsets

Subject	Untreated T cells		OKT4-enriched		OKT8-enriched	
	nmol/h per 10^6 cells*	% †	nmol/h per 10^6 cells*	% †	nmol/h per 10^6 cells*	% †
Controls						
1	6.56	—	3.34	—	13.5	—
2	12.6	17	6.42	15	18.3	30
3	12.3	28	6.80	13	17.4	29
4	12.2	22	8.0	16	15.6	33
5	9.70	—	4.09	—	15.9	—
5 (Repeated)	11.5	—	3.59	—	10.2	—
6	11.6	32	5.80	11	29.0	47
Mean \pm 1 SD	10.9 ± 2.1	25 ± 7	5.43 ± 1.8	14 ± 2	17.1 ± 5.9	35 ± 8
Patients						
1 CAG	5.26	12	3.08	12	7.86	10
2 CAG	5.42	4	1.57	12	8.53	4
6 CVI	4.29	8	3.09	4	5.98	8
7 CVI	3.27	12	3.66	12	4.90	8
Mean \pm 1 SD	4.56 ± 0.99	9.0 ± 4	2.85 ± 0.90	10 ± 4	6.82 ± 1.7	7.5 ± 3

* Ecto-5'-NT activity based upon the quantitative radiochemical assay.

† Percentage of cells positive for ecto-5'-NT activity by histochemical stain.

in OKT4- or OKT8-enriched subpopulations when compared with untreated control T cells or T cells treated with OKT6 + C'.

Helper and suppressor activity of patients' T cells toward *in vitro* immunoglobulin biosynthesis. In eight separate experiments, equal numbers of B cells and irradiated (2,000 rad) T cells (5×10^5 cells of each) from control subjects synthesized $14,800 \pm 6,400$ ng of IgM and $11,000 \pm 4,000$ ng of IgG after 7 d of culture in the presence of PWM. B cell preparations were sufficiently free of T cells that B cells alone + PWM synthesized <10% of the IgM and IgG made by equal numbers of B cells + irradiated T cells. Helper activity of the patients' T cells was assessed by comparing the quantities of IgM and IgG synthesized by control B cells + irradiated control T cells with that synthesized by control B cells + irradiated patient T cells. In all seven patients studied, the patients' irradiated T cells supported $\geq 65\%$ of the level of IgM and IgG synthesized by control B cells + irradiated control T cells (Table III). Thus none of the patients had defective helper-T cell activity.

Suppressor activity of the patients' T cells was assessed by comparing the quantities of IgM and IgG synthesized by co-cultures of patients and control PBM (5×10^5 cells of each) in response to PWM with the quantities of IgM and IgG synthesized by individual cultures of patient PBM and control PBM as described by Waldmann (40). In three of the seven patients studied (9–11) co-cultures of patient and control PBM synthesized >70% of the expected quantities of IgM and IgG (Table III). These patients were judged to have normal T suppressor activity. In the other four patients (2, 4, 6, 8), however, the quantities of IgM and IgG

synthesized in co-cultures was suppressed by 74 to >95% as compared to that synthesized in individual cultures of patient and control PBM. Thus, these four patients were considered to have increased suppressor T cell activity. In all four cases, additional co-culture experiments with purified T cells showed that the increased suppressor activity was due to radiation-sensitive (2,000 rad) T cells.

The patients' T cell ecto-5'-NT activity was compared with our evaluation of their T helper and suppressor activity toward *in vitro* immunoglobulin biosynthesis. The three patients with normal T cell ecto-5'-NT activity (9–11) had normal helper and suppressor function, while all four patients with low T cell ecto-5'-NT activity (2, 4, 6, 8) had increased T suppressor activity.

DISCUSSION

Ecto-5'-nucleotidase is a cell surface enzyme that is present on only a subpopulation (~25%) of normal human T cells. This subpopulation includes both helper and suppressor T cells as defined by the monoclonal antibodies OKT4 and OKT8, respectively. By quantitative radiochemical assay, ecto-5'-NT activity in OKT4⁺-enriched preparations isolated from six normal donors was 5.43 ± 1.8 nmol/h per 10^6 cells with $14 \pm 2\%$ of cells positive by histochemical stain; that in OKT8⁺-enriched populations was 17.1 ± 5.9 nmol/h per 10^6 cells with $35 \pm 8\%$ of cells positive by histochemical stain. Although OKT8⁺ cells are enriched for ecto-5'-NT activity, the activities of ADA and PNP, two additional enzymes of the purine salvage pathway, are approximately equal in OKT4⁺ and OKT8⁺ cells.

TABLE III
Helper and Suppressor Activity of Patient Lymphocytes toward PWM-driven *In Vitro* Immunoglobulin Biosynthesis

Patient	B _{control} + T _{patient} (irradiated)*		PBM _{patient}		PBM _{control}		PBM _{patient} + PBM _{control} †	
	IgM	IgG	IgM	IgG	IgM	IgG	IgM	IgG
	ng synthesized/ 10^6 B cells				ng synthesized/ 10^6 PBM			
2	20,100 (>100)§	26,700 (>100)§	<5	<5	6,316	1,673	<5 (<5)	<5 (<5)
4	11,300 (72)	6,000 (69)	<5	<5	3,787	1,594	<5 (<5)	<5 (<5)
6	11,800 (72)	8,400 (71)	576	267	6,590	6,725	690 (19)	902 (26)
8	12,000 (65)	10,100 (80)	58	5	4,440	1,890	18 (5)	19 (<5)
9	11,800 (74)	4,900 (70)	317	7	5,824	3,315	3,024 (98)	1,741 (105)
10	12,600 (82)	10,700 (79)	581	267	2,918	4,036	1,246 (71)	1,755 (82)
11	11,300 (85)	9,800 (75)	412	119	3,617	2,481	1,622 (81)	1,746 (134)

* In eight separate experiments, B_{control} + T_{control} (irradiated) synthesized $14,800 \pm 6,400$ ng IgM and $11,000 \pm 4,000$ ng IgG.

§ Percentage of IgM and IgG synthesized by B_{control} + T_{control} (irradiated).

† In 16 separate experiments, PBM_{control A} + PBM_{control B} gave $104 \pm 41\%$ of the expected IgM synthesized and $111 \pm 32\%$ of the expected IgG.

^{||} Percentage of the expected IgM or IgG synthesized.

We have previously measured ecto-5'-NT activity in helper ($T\mu$) and suppressor ($T\gamma$) T cells as defined by Fc receptors, according to Moretta et al. (41, 42). We found $T\mu$ cells to have threefold more ecto-5'-NT activity than $T\gamma$ cells (21) (11.9 ± 1.6 vs. 4.5 ± 1.3 nmol/h per 10^6 cells). These results clearly show that Fc γ and Fc μ receptors and the OKT monoclonal antibodies do not define identical subsets of helper and suppressor T cells. Indeed, it is well-documented (30, 32, 33, 43) that $T\mu$ cell preparations contain cells recognized by OKT4 and OKT8, while $T\gamma$ cells are predominantly recognized by monoclonal antibody OKM1.

Ecto-5'-NT activity was significantly reduced (<5.5 nmol/h per 10^6 cells as compared to 10.1 ± 3.5 nmol/h per 10^6 cells for control subjects) in total T cell preparations from all four patients with CAG and three of seven patients with CVI, confirming both our earlier work (17) and the work of Edwards (23) and Webster (24). In no case could low T cell ecto-5'-NT activity be explained by increased proportions of T cell subpopulations normally low in ecto-5'-NT activity (i.e., $T\gamma$ and OKT4⁺ T cells). All of the patients studied had normal or even decreased percentages of $T\gamma$ cells; two of four patients with CAG and four of seven patients with CVI had decreased percentages of OKT4⁺ T cells and corresponding increases in percentages of OKT8⁺ T cells to yield OKT4/OKT8 ratios of 0.60 to 1.0 (as compared to 1.7 ± 0.2 for control subjects), similar to those reported by Reinherz et al. (44). In four patients, low total T cell ecto-5'-NT activity was due to reduced numbers of ecto-5'-NT positive cells, especially in the OKT8⁺ population, rather than to substantially less ecto-5'-NT activity per cell. In OKT4⁺-enriched preparations from these patients, ecto-5'-NT activity was 2.85 ± 0.90 nmol/h per 10^6 cells with $10 \pm 4\%$ of cells positive by histochemical stain; in OKT8⁺-enriched preparations it was 6.82 ± 1.7 nmol/h per 10^6 cells with $7.5 \pm 3\%$ of cells positive. These results confirm and extend the earlier work of Matamoros et al. (45) and Recker et al. (46) who found decreased numbers of ecto-5'-NT positive PBM and T cells in patients with CAG and CVI.

The mechanism responsible for altered proportions of ecto-5'-NT positive and negative T cells in hypogammaglobulinemia is unknown. It is, however, unlikely to be secondary to chronic infections since it was found in patient 3 at birth and in patient 8 who is in good health. Ecto-5'-NT deficiency cannot be a consequence of immunoglobulin replacement therapy (as has been suggested for decreased PWM responses in nonimmunodeficient children treated with gammaglobulin [47]), since it was detected in two of our patients before the initiation of treatment. Although ecto-5'-NT activity is a marker for T lymphocyte differentiation (22, 23, 48), with thymocytes having vir-

tually absent activity, it is unlikely that low numbers of T cells with ecto-5'-NT activity in CAG and CVI patients can be explained merely by a block in T cell maturation. The T cells of none of our patients reacted with the monoclonal antibodies OKT10 or OKT6 (data not shown), both of which recognize thymocyte antigens (34). Furthermore, none of the patients suffered from a clinical deficit in T cell function as would be expected with a predominance of immature T lymphocytes.

In preliminary studies with seven patients, increased proportions of ecto-5'-NT negative T cells correlated with increased suppressor T cell activity toward PWM-driven in vitro immunoglobulin biosynthesis. These results suggest that human suppressor T cells belong to the ecto-5'-NT negative subpopulation. Confirmation of this hypothesis awaits functional studies on purified preparations of ecto-5'-NT positive and negative T cells and unfortunately no methods are yet available for performing these separations. It is important to note that the proportions of T cells recognized by monoclonal antibodies OKT4 and OKT8 did not always correctly predict the functional defects in our patients' T cells. Patients 2 and 8 had relatively normal ratios of OKT4⁺ to OKT8⁺ T cells, yet showed increased suppressor activity; patient 11 had a distinctly abnormal OKT4⁺/OKT8⁺ ratio, yet normal helper and suppressor activity. These results are consistent with the accumulating evidence that although the monoclonal antibodies OKT4 and OKT8 define T cell subsets enriched for help and suppression, respectively, of PWM-driven immunoglobulin synthesis in healthy adult donors (25, 49), the antigens recognized by these antibodies do not always correlate with function during development or in some patients with immunodeficiency disease. In cord blood (50), suppression is found in the OKT4⁺, rather than the OKT8⁺ population and Reinherz recently (51) described a patient with a functional helper T cell defect, but normal numbers of OKT4⁺ cells. There is also increasing evidence that OKT4⁺ and OKT8⁺ cells are heterogeneous with respect to both function and cell surface determinants (50-54). Our data demonstrate that ecto-5'-NT activity defines a subpopulation of T cells that overlaps the subpopulations defined by OKT4 and OKT8 and that the proportions of ecto-5'-NT positive and negative T cells is markedly altered in many patients with CAG and CVI. Since increased numbers of ecto-5'-NT negative cells appear to correlate with enhanced suppressor activity in the seven patients examined in our studies, ecto-5'-NT activity may define functional subsets of T cells and thus prove to be a useful T cell surface marker in the study of regulatory T cell subset imbalances leading to hypogammaglobulinemia.

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