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Subpopulations of human T lymphocytes

XVIII. T LYMPHOCYTES WITH RECEPTORS FOR IgE (Tε) IN PATIENTS WITH PRIMARY IMMUNODEFICIENCY AND HYPERIMMUNOGLOBULINAEMIA E STATES

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SUMMARY

Four of 18 patients with common variable immunodeficiency and one of eight patients with selective IgA deficiency had increased proportions of Tε cells. Three patients with common variable immunodeficiency and two with selective IgA deficiency had increased numbers of Tε cells. Three patients with common variable immunodeficiency had decreased numbers of Tε cells. Five of six patients with hyperimmunoglobulinaemia E had high proportions of Tε cells; this increase was not related to the elevated IgE levels.

INTRODUCTION

Human and rat T and B lymphocytes have receptors for IgE Fc (Gonzalez-Molina & Spiegelberg, 1977; Hellstrom & Spiegelberg, 1979; Yodoi & Ishizaka 1979a) which are increased in *Nippostrongylus brasiliensis* (Nb) infected rats, probably because of increased concentration of IgE (Yodoi, Ishizaka & Ishizaka, 1979). In rats, Tγ cells change to Tε cells in the presence of high concentrations of IgE (Yodoi & Ishizaka, 1979b); therefore, these investigators concluded that the increase in Tε cells in infected rats does not involve recruitment of Tγ cells to Tε cells. Furthermore, it appears that the binding of IgE with either Tγ or Tε stimulates lymphocytes to form Fcε receptors. The present study was undertaken to examine the proportion and number of Tε cells in the blood from patients with hyper IgE states and a variety of primary immunodeficiency disorders and to study their relationship with the levels of serum IgE.

MATERIALS AND METHODS

Twenty-eight patients with primary immunodeficiency disorders (13–45 years of age), classified according to WHO recommendations (Cooper *et al.*, 1973), included 18 patients with common variable immunodeficiency, eight with selective IgA deficiency and two with x-linked congenital agammaglobulinaemia. Three of six patients with hyper IgE states were atopic; the remaining three were classified as hyper IgE syndrome (Buckley, 1979). Thirty healthy age- (18–42 years) and sex-matched donors served as controls.

Mononuclear cells were isolated from heparinized peripheral venous blood on Ficoll–Hypaque (FH) gradients. Cells were washed three times in Hanks' balanced salt solution (HBSS) and resuspended in medium RPMI 1640 (Grand Island Biological Co., Grand Island, New York) containing 20% heat-inactivated fetal calf serum (FCS) at a concentration of 4×10^6 cells/ml. Total T cells in an aliquot were counted by spontaneous rosette formation with sheep RBC and

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phagocytic cells were recognized by ingestion of 0·8-μm-size polystyrene latex particles (Dow Chemicals, Indianapolis, Indiana). Cells were washed and resuspended in HBSS at 4×10^6/ml.

To isolate T lymphocytes, mononuclear cells suspended in medium containing FCS at 4×10^6/ml were mixed with carbonyl iron (Lymphocyte Separator Reagent, Technicon, Tarrytown, New York) at a ratio of 2:1 (v/v) and incubated at 37°C for 30 min on a rotator and phagocytic cells were separated on FH density gradient by centrifugation at 400 *g* for 20 min. Lymphoid cells from the interface were washed three times with HBSS and resuspended to a concentration of 4×10^6/ml. T cells were isolated from non-T lymphocytes by rosetting with SRBC and separating on FH gradient by a method described (Gupta *et al.*, 1978). T cells were washed three times with HBSS, resuspended in RPMI 1640 at a concentration of 3×10^6/ml and analysed for Tε cells. T cells were more than 97% purified. Viability of the purified T cells was more than 98%.

To prepare IgE-coated erythrocytes (EAε), OxRBC were coated with ovalbumin using the chromium chloride technique (Gupta, Platsoucas & Good, 1979a). One per cent OxRBC coated with ovalbumin were incubated with an appropriate dilution (determined by titration) of murine monoclonal IgE from the murine hybrid cell line IgE-14-205 (kindly provided by Dr I. Bottcher of Schering AG, Berlin) which has anti-ovalbumin specificity.

To count Tε cells, 100 μl of purified T cell suspension (3×10^6/ml) were mixed with an equal volume of 1% EAε centrifuged at 200 *g* for 5 min and incubated on ice for 1 hr. The pellet was gently resuspended and 500 cells were counted for rosette formation. A lymphocyte with three or more RBC attached was considered a rosette. T cells mixed with OxRBC coated with ovalbumin always gave a background count of less than 0·1%.

Results are expressed as a percentage of total T cells. Ranges for T cells and Tε cells are based on the ranges observed simultaneously in 30 healthy controls and were not transformed to logs.

RESULTS

Only one of the eight patients with selective IgA deficiency had a high proportion of Tε cells but the numbers of Tε cells were increased in two (Table 1). Three had a low proportion of total T cells, including two with high Tε cells, but absolute numbers of total T cells were reduced in only one patient. Patients with increased Tε cells were found to have either normal or low serum IgE levels.

Table 1. Tε cells and serum immunoglobulin E in patients with x-linked infantile agammaglobulinaemia and selective immunoglobulin A deficiency

Patient No.	Total T (%)	Total T (per mm^3)	Tε (%)	Tε (per mm^3)	Serum IgE (iu)
x-linked agammaglobulinaemia					
1	79·0	606†	2·0	15	< 5
2	75·0	369†	2·0	10	< 5
Selective IgA deficiency					
1	69·0	1,711	3·0	74	54
2	66·0	1,729	1·0	26	< 5
3	50·0†	943†	5·0*	94*	15
4	82·0	2,033	2·0	49	32
5	44·0†	1,258	3·0	860*	< 5
6	79·0	1,807	2·0	45	750*
7	65·0	1,998	1·0	31	36
8	60·0†	1,200	0·5	10	20
Controls (range)	65–90	1,105–2,800	0·5–5	10–80	25–250

* Abnormally high.
† Abnormally low.

One patient with selective IgA deficiency (patient 6) had high levels of serum IgE (750 iu), but the proportion and number of Tε cells were within the range found for healthy controls. The proportion and number of Tε cells were uniformly distributed between this age group of healthy controls. Both patients with x-linked infantile agammaglobulinaemia had a normal number and proportion of Tε cells and both had relatively low numbers of T cells.

Four of 18 patients with common variable immunodeficiency had a high proportion of Tε cells when compared to age- and sex-matched controls; however, the absolute numbers of Tε were increased in only three (Table 2). All but one with increased proportions and numbers of Tε cells had low levels of serum IgE. Three patients had decreased numbers of Tε cells. Four of 19 patients had low proportions and 11 patients had low numbers of total T lymphocytes.

Table 2. Tε cells and serum immunoglobulin E in patients with primary immunodeficiency

Patient No.	Total T (%)	Total T (per mm^3)	Tε (%)	Tε (per mm^3)	Serum IgE (iu)
Common variable immunodeficiency					
1	83·0	2,838	0·9	31	< 5
2	43·0†	423†	0·5	5†	< 5
3	67·0	630†	3·0	28	< 5
4	87·0	584†	6·0*	40	< 5
5	71·0	469†	2·0	13	< 5
6	38·0†	1,162	1·0	31	< 5
7	65·0	786†	3·0	36	< 5
8	56·0†	672†	3·0	36	< 5
9	73·0	1,027†	6·0*	84*	< 5
10	86·0	1,170	4·0	54	< 5
11	72·0	933†	1·0	13	< 5
12	43·0†	1,263	1·0	29	200
13	78·0	823†	0·5	5†	< 5
14	84·0	1,179	2·0	28	< 5
15	73·0	1,460	5·5*	110*	260*
16	78·0	1,245	9·0*	112*	< 5
17	83·0	892†	4·0	43	< 5
18	74·0	543†	0·5	4†	0
Controls (range)	65–90	1,105–2,800	0·5–5·0	10–80	5–250

* Abnormally high.
† Abnormally low.

Table 3. Tε cells and serum immunoglobulin E in patients with hyperimmunoglobulinaemia E

Patient No.	Total T (%)	Total T (per mm^3)	Tε (%)	Tε (per mm^3)	Serum IgE (iu)
1	80·0	3,184	6·0*	238*	620
2	87·0	1,153	6·0*	79	470
3	55·0†	891†	6·0*	97*	540
4	53·0†	821†	22·0*	341*	480
5	79·0	1,580	15·0*	541*	700
6	60·0†	2,332	0·5	19	2,200
Controls (range)	65–90	1,105–2,800	0·5–5·0	10–80	5–250

* Abnormally high.
† Abnormally low.

Five of six patients with hyperimmunoglobulinaemia E states had increased proportions and four of six had increased numbers of Tε cells (Table 3). The levels of serum IgE did not correlate with the proportion or number of Tε cells. Three of six patients had low proportions and two of six had decreased numbers of total T cells.

DISCUSSION

Some murine and human T cells (Tε) have receptors for IgE Fc (Yodoi & Ishizaka, 1979a); these cells are distinct from T cells with IgG Fc receptors (Tγ). However, a large proportion of activated T cells in rats possess receptors for both IgG Fc and IgE Fc. Yodoi *et al.* (1979) reported a high proportion of Tε cells in rats infected with *Nippostrongylus brasilienis* (Nb) which is apparently due to Tγ cells changing to Tε cells in the presence of high concentrations of IgE in the serum or *in vitro*. These changes do not involve recruitment of Tγ cells to Tε cells. It appears that the binding of IgE to Fc receptors of either Tγ or Tε cells stimulates them to form Fcε receptors. These authors suggested that Tε cells play an immunoregulatory role in IgE responses.

In mice it has been shown that both antigen-specific and non-specific T lymphocytes exert a regulatory control on the reaginic antibody responses (Tada, 1975; Katz, 1978; Chiorazzi, Fox & Katz, 1977). Patients with primary immunodeficiency disorders often have increased levels of serum IgE. Patients with primary immunodeficiency and allergic disorders may have an imbalance of the Tμ and Tγ cell subpopulations (Gupta & Good, 1977, 1978; Moretta *et al.*, 1977a; Trompeter, Layward & Hayward, 1978; Canonica *et al.*, 1979; Gupta, Fikrig & Good, 1980). Tμ cells may have helper activity and Tγ cells suppressor activity (Moretta *et al.*, 1977b); however, neither of these populations are homogeneous in their immunoregulatory property (Hayward *et al.*, 1978; Gupta, Schwartz & Good, 1979b; Gupta & Good, 1980). Most of our patients with hyper IgE states had increased proportions and numbers of Tε cells, whether they were atopic or had the hyperimmunoglobulinaemia E syndrome. There was no correlation between the number or proportion of Tε cells and the levels of serum IgE. One patient with hyper IgE syndrome and one with primary immunodeficiency who had increased IgE levels had normal proportions and numbers of Tε cells. In contrast, six patients with primary immunodeficiencies had increased proportions and numbers of Tε cells but had normal or low levels of serum IgE. These observations suggest that serum IgE is not the sole factor responsible for increased numbers or proportions of Tε in patients with primary immunodeficiency and hyper IgE states. There may be at least two subpopulations of Tε cells, a helper and the other a suppressor for IgE responses. The proportion of Tε cells in our healthy controls was higher than that reported by Yodoi & Ishizaka (1979b). They did not find rosette formation between human T cells and red cells coated with rat IgE myeloma, as we did using mouse IgE monoclonal antibody.

Studies are in progress to understand the regulatory role of purified human Tε cells in IgE responses.

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