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Subpopulations of human T lymphocytes

I. STUDIES IN IMMUNODEFICIENT PATIENTS

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SUMMARY

T lymphocytes with receptors for IgM(Tμ) and IgG(Tγ) were examined in thirty patients with primary immunodeficiency and autoimmune disorders. Six out of twenty-seven patients with primary immunodeficiency had a low proportion of Tμ cells when compared with normal controls. Eight out of twenty-seven patients with primary immunodeficiency had an increased proportion of Tγ cells. Two out of twenty-seven patients had both a low proportion of Tμ cells and a high proportion of Tγ cells. The patient studied with severe combined immunodeficiency had a low proportion of both Tμ and Tγ cells. Patients with Bruton-type agammaglobulinaemia, common variable immunodeficiency, thymoma and immunodeficiency syndrome and selective IgA deficiency demonstrated heterogeneity with regard to alterations in T-cell subpopulations.

INTRODUCTION

The study of lymphocyte subpopulations has been instrumental in classifying and understanding some of the basic defects in patients with a variety of primary and secondary immunodeficiency diseases. T cells are characterized by their spontaneous rosette formation with sheep erythrocytes (SRBC) and by the presence at the cell surface of T-cell antigen (HTLA) (Jondal, Holm & Wigzell, 1972; Ablin & Morris, 1973; Touraine *et al.*, 1974). B lymphocytes are identified by the presence of surface immunoglobulins (Rabellino *et al.*, 1971) and receptors for IgG Fc (Dickler & Kunkel, 1972), complement components (Ross *et al.*, 1973) and mouse erythrocytes (Stathopoulos & Elliott, 1974; Gupta & Grieco, 1975; Gupta, Good & Siegal, 1975; Gupta, Good & Siegal, 1976a). Third population cells (non-T and non-B cells) are detected by the presence of high-avidity IgG Fc receptors, but they lack surface immunoglobulins, phagocytic capacity and T-cell characteristics (Froland, Wisloff & Michaelsen, 1974). Recently, human T lymphocytes have been shown to express receptors for rabbit IgG (Tγ) (Ferrarini *et al.*, 1975; Moretta *et al.*, 1976a) and IgM (Tμ) (Gmelig-Myeling, Van Der Ham & Ballieux, 1976; Moretta *et al.*, 1975, 1976b; McConnell & Hurd, 1976). Moretta *et al.* (1977) have demonstrated that Tμ cells act as helpers for the proliferation and differentiation of B cells to plasma cells, and activated Tγ cells act as suppressor cells. Waldmann *et al.* (1976) and Siegal, Siegal & Good (1976) have recently shown that certain patients with primary and secondary immunodeficiency disorders have increased suppressor cell activity in their circulating leucocytes. The purpose of the present investigation was to study the distribution of Tμ and Tγ cells in patients with a variety of primary immunodeficiency and autoimmune disorders.

MATERIALS AND METHODS

Twenty-seven patients with primary immunodeficiency were classified according to WHO recommendations (Cooper *et al.*, 1973) and included patients with Bruton-type agammaglobulinaemia (nine), severe combined immunodeficiency (one), common variable immunodeficiency (ten), thymoma and immunodeficiency syndrome (two) and selective IgA deficiency (five). Three patients with autoimmune disease included patients with systemic lupus erythematosus (two) and mixed cryoglobulinaemia (one). Thirty healthy well-screened blood bank donors served as controls. Absolute lymphocyte counts

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in the patient group were comparable to those of control groups with the exception of one patient who had severe combined immunodeficiency and was lymphopenic.

Isolation of mononuclear cells. Mononuclear cells were isolated on Ficoll–Hypaque (FH) density gradient (Böyum, 1958). Cells were washed three times in phosphate-buffered saline (PBS) and resuspended in Hanks' balanced salt solution (HBSS) to a desired concentration. More than 98% of the cells were viable as tested by the trypan blue dye exclusion test. Phagocytic cells were removed by permitting them to ingest carbonyl iron and removing them with a powerful magnet (Lundgren, Zukoski & Moller, 1968). This procedure did not change the relative proportions of lymphocyte subpopulations. Less than 1% peroxidase-positive cells were seen in purified preparations. These monuclear cells were examined for T, B and third population cells. Study of Tμ and Tγ was carried out on a population of purified T lymphocytes.

T lymphocytes (SRFC). 100 μl of lymphocyte suspension (5×10^6 cells) were mixed with 25 μl of human AB serum (heat-inactivated and absorbed with SRBC) and 100 μl of 0·5% SRBC. The mixture was then incubated at 37°C for 5 min and centrifuged at 50 *g* for 5 min followed by incubation at 4°C for 18 hr. The pellet was gently resuspended and 200 lymphocytes were counted for rosette formation.

B lymphocytes. B cells were determined for the presence of surface immunoglobulins, using fluorescein-conjugated monospecific antisera against μ, α, and γ determinants of immunoglobulins (Rabellino *et al.*, 1971). 25 μl of antiserum was mixed with 10^6 cells per tube and incubated at 4°C for 30 min. Cells were washed three times with 2% bovine serum albumin in PBS (BSA–PBS) and resuspended in a minute volume of BSA–PBS (25 μl). Wet mounts were prepared and 200–300 lymphocytes counted using a Leitz orthoplan fluorescence microscope equipped with epi-illumination. The percentages of B lymphocytes were expressed as the sum of per cent of lymphocytes with surface IgM, IgG or IgA.

Third population cells (Ripley rosette). 100 μl of lymphocyte suspension (5×10^6 cells per ml) were mixed with 100 μl of 1% human O Rh+ erythrocytes sensitized with Ripley serum (containing high titres of 7S IgG anti-D human RBC antibody) and were centrifuged at 200 *g* for 5 min followed by incubation at 4°C for 30 min. The pellet was resuspended and 200 lymphoid cells counted for rosette formation. A cell with four or more red cells attached was considered a rosette.

Determination of T lymphocyte subpopulations. Purification of T lymphocytes. T lymphocytes were purified from non-T cells by rosetting with SRBC, as described above, with a minor modification: instead of an 18 hr incubation, cells were incubated for 1 hr with 1% neuraminidase-treated SRBC and separated from non-rosetting (non-T) cells on a FH gradient. SRBC attached to T cells were lysed by using Tris buffer with 0·83% ammonium chloride. T lymphocytes obtained in this way were more than 98% pure, as determined by rosette formation with SRBC and lack of surface immunoglobulin. Cells were washed three times with PBS and resuspended in medium RPMI 1640 (Grand Island Biological Co., Grand Island, N.Y.) containing penicillin (100 u/ml), streptomycin (100 μg/ml) and 20% foetal calf serum to a concentration of 2×10^6 cells per ml. These cells were cultured at 37°C in a humidified incubator with 5% CO_2 and 95% air for 20 hr. At the end of incubation cells were washed three times in PBS and resuspended to a concentration of 4×10^6 per ml. More than 98% of cells were viable as tested by trypan blue dye exclusion. Cells were then assayed for Tμ and Tγ subpopulations.

Anti-ox RBC antibodies. Anti-ox RBC antibodies were raised in rabbits by a primary injection of 2 ml of 10% ox RBC emulsified in Freund's complete adjuvant. Booster injections were given at weekly intervals. The first bleeding was taken 1 week following the first immunization. The late bleedings were taken 2, 3 and 4 weeks after the first immunization, and were pooled. IgM and IgG were prepared from early and late bleedings by DEAE–cellulose chromatography and sephadex G-200 gel filtration. Both fractions were concentrated by 50% ammonium sulphate precipitation. After dialysis against PBS, each fraction was adjusted to a haemolytic titre of 1/1000. Rabbit IgM and IgG anti-ox RBC gave a single precipitation line in immunoelectrophoresis against rabbit anti-immunoglobulin antisera.

Preparation of ox RBC–antibody complexes. Ox RBC were washed three times in PBS and resuspended to a concentration of 2%. Equal volumes of ox RBC and purified IgM antibody (1/250 dilution) and IgG antibody (1/100 dilution) were mixed and incubated at room temperature for 90 min. Following incubation, cells were washed three times in PBS and resuspended to a concentration of 1·0%. Ox RBC with IgM(EA_M) and IgG(EA_G) were prepared fresh each time.

T cells with receptors for IgM (Tμ). 100 μl of T lymphocyte suspension were mixed with 100 μl of EA_M and centrifuged at 200 *g* for 5 min followed by incubation at 4°C for 30 min. The pellet was resuspended and 200 lymphocytes were counted for rosette formation. A lymphocyte with three or more red cells attached was considered a rosette.

T cells with receptors for IgG (Tγ). 100 μl of T lymphocyte suspension were mixed with 100 μl of EA_G and centrifuged at 200 *g* for 5 min, followed by incubation at 4°C for 30 min. The pellet was resuspended and 200 lymphocytes were counted for rosette formation. A rosette was defined as a lymphocyte with three or more red cells attached.

The results of Tμ and Tγ cells are presented as percentage of T lymphocytes.

RESULTS

Results of studies of subpopulations of T cells are shown in Table 1. All patients with Bruton-type agammaglobulinaemia had less than 0·5% B cells and normal proportions of third population cells. Two patients (K.W. and R.W.), both females with findings otherwise like those of Bruton-type agammaglobulinaemia (Hoffman *et al.*, 1977), had a higher proportion of T lymphocytes (94·5% and 96%). The patient (G.B.), a male with X-linked disease, had a low proportion of Tμ cells (31·0%) and a normal

proportion of Tγ cells. The patient (R.M.) had a normal proportion of Tμ cells, and increased proportions of Tγ cells. The two female patients (K.W. and R.W.) had a normal proportion of Tμ cells and an increased proportion of Tγ cells (48% and 45%). The other patients had a normal proportion of both Tμ and Tγ cells.

TABLE 1. T-lymphocyte subpopulations in immunodeficient patients

Patients	SRFC (%)	Tμ (%)	Tγ (%)	Third population cells (%)	B lymphocytes (%)
Bruton-type agammaglobulinaema					
R.M.	81·0	37·5	21·5	18·0	0·0
G.B.	82·0	31·0	9·5	16·0	0·0
D.B.	81·5	79·0	5·0	15·0	0·0
R.A.	90·0	58·0	9·0	9·0	< 0·5
I.A.	84·5	79·5	3·5	12·5	< 0·5
M.R.	88.0	57·5	7·0	10·5	0·0
N.P.	86·0	75·0	3·5	12·0	0·0
K.W.	94·5	60·0	45·0	5·0	< 0·5
R.W.	96·0	59·0	48·0	14·0	< 0·5
Common variable immunodeficiency					
C.V.	89·0	21·0	25·0	10·0	7·0
C.B.	83·0	75·0	3·0	6·0	6·5
R.R.	91·0	30·0	6·0	6·5	6·5
S.P.	52·5	47·0	9·0	11·0	35·0
M.R.	80·0	64·0	6·0	8·0	10·0
E.C.	78·5	57·0	16·0	16·0	6·0
J.M.	84·0	35·0	6·0	12·0	6·0
T.S.	86·0	45·5	6·5	14·0	7·0
D.A.	78·5	19·0	3·0	9·0	10·5
M.E.	89·0	62·0	5·0	12·0	5·5
Thymoma and immunodeficiency					
W.S.	75·5	21·0	25·0	22·0	0·0
F.K.	84·0	51·0	22·0	10·0	0·0
Selective IgA deficiency					
E.H.	80·5	43·5	3·0	11·0	7·0
J.G.	82·0	78·0	2·0	7·0	7·5
S.V.	85·0	53·0	22·0	12·5	10·5
R.N.	80·0	50·0	6·0	10·5	7·5
T.S.	84·0	64·0	5·0	10·0	10·0
Severe combined immunodeficiency					
B.H.	12·0	1·5	1·5	7·0	80·0
Systemic lupus erythematosus					
I.R.	78·0	57·0	1·0	10·0	12·0
J.Z.	84·0	66·0	1·0	6·0	14·0
Mixed cryoglobulinaemia					
G.D.	72·0	59·0	10·0	20·0	8·0
Controls* (n = 30)	(65–93)	(35–75)	(3–15)	(5–24)	(2–20)

* Control ranges are shown.

One (S.P.) out of eleven patients with common variable immunodeficiency had a low proportion of T cells and a high proportion of B cells. Other patients (C.V., R.R. and D.A.) had a low proportion of Tμ cells (21%, 30·5% and 19·0%). Two patients (C.V. and E.C.) had high a proportion of Tγ cells (25% and 16%), whereas the other patients had a normal proportion of both Tγ and Tμ cells.

Both of the patients with thymoma and immunodeficiency demonstrated a high proportion of Tγ cells. Only one of them (W.S.) had a low proportion of Tμ cells. Both the patients lacked B lymphocytes and had a normal proportion of third population cells and T cells.

One patient with severe combined immunodeficiency had a low proportion of T lymphocytes and an increased proportion of B lymphocytes. The proportions of both Tμ and Tγ cells were low.

One patient with selective IgA deficiency (S.V.) had a normal proportion of Tμ cells and an increased proportion of Tγ cells. The other four patients had normal proportions of both Tμ and Tγ cells.

Both patients with systemic lupus erythematosus had a normal proportion of Tμ cells but a lower than normal proportion of Tγ cells. A patient with mixed cryoglobulinaemia had normal proportions of both Tμ and Tγ cells.

DISCUSSION

The present study demonstrated in normal persons a large proportion of T lymphocytes with receptors for IgM (Tμ cells). Similar results have been reported by other investigators (Gmeling-Myeling *et al.*, 1976; Moretta *et al.*, 1975, 1976b; McConnell & Hurd, 1976). Brochier, Samarut & Revillard (1975) found no rosette formation of human T lymphocytes with chicken erythrocytes coated with IgM antibody. The receptors for IgG (Tγ cells) were present on a relatively small proportion of T lymphocytes. Moretta *et al.* (1976b) and Ferrarini *et al.* (1975), using a similar system, found a comparable proportion of Tγ cells to those reported here. Samarut, Brochier & Revillard (1976) using chicken erythrocytes coated with IgG antibody found 7% of human T lymphocytes to have IgG Fc receptors. IgG Fc receptors have also been demonstrated on murine, guinea-pig and chicken thymocytes and T lymphocytes (Grey, Kubo & Cerottini, 1972; Santana & Turk, 1975; Van Boxel & Rosenstretch, 1974; Anderson & Grey, 1974; Gyongyossy *et al.*, 1975). The present study and that of Moretta *et al.* (1975) and McConnell & Hurd (1976) required prior incubation of T lymphocytes *in vitro* for 15–20 hr at 37°C for the full expression of receptors for IgM. Gmelig-Myeling *et al.* (1976) could demonstrate 46% of human T lymphocytes reveal receptors for IgM without prior incubation; however, we found a much smaller proportion (5–10%) of T lymphocytes with IgM receptor when we did not use the incubation step (unpublished data).

Patients with Bruton-type agammaglobulinaemia usually lack circulating B lymphocytes; however, they have a normal proportion of third population cells and a normal or increased proportion of T lymphocytes (Gupta, 1976; Gupta & Good, 1976; Gupta, Good & Siegal, 1976b; Gajl-Peczalska *et al.*, 1973; Cooper & Lawton, 1972). All the patients studied here lacked circulating B lymphocytes almost completely and had a normal proportion of third population cells. Two patients had a high proportion of T cells. Three out of nine patients had an increased proportion of Tγ cells and a normal proportion of Tμ cells. One patient with X-linked disease had a normal proportion of Tγ cells and a low proportion of Tμ cells. Waldmann *et al.* (1976) and Siegal, Siegal and Good (1976) have demonstrated increased suppressor T-cell activity in certain patients with Bruton-type agammaglobulinaemia. This may be an important relationship since Moretta *et al.* (1977) have shown that immune complex-induced non-specific suppressor lymphocyte activity in the pokeweed mitogen system can be related to Tγ cells. Further studies comparing spontaneous suppressor activity and numbers of Tγ cells seem indicated.

Patients with severe combined immunodeficiency are characterized by a paucity of both T and B lymphocytes (Cooper *et al.*, 1973; Hayward & Greaves, 1975); however, certain cases with increased relative proportions of B lymphocytes have been reported (Cooper & Lawton, 1972; Preud'homme, Clauvel & Seligmann, 1975). Our patient also represents a category of severe combined immunodeficiency in which high proportions of B cells and low proportions of T cells are found. Both Tμ and Tγ cells were present in very low proportions. The hypogammaglobulinaemia seen in some of these patients may result from lack of helper (Tμ) cells rather than increased suppressor cell activity.

Patients with immunodeficiency and thymoma represent a combined cell-mediated and antibody-mediated immunodeficiency that appears late in life. These patients usually lack B cells entirely or have very low proportions of B cells (Gupta, 1976; Gupta & Good, 1976; Gupta *et al.*, 1976b). Both of our patients had normal proportions of total T cells and third population cells. B lymphocytes were completely lacking. Both of the thymoma–agammaglobulinaemia patients were distinct in their immunological profile. One (F.K.) had panhypogammaglobulinaemia while the other (W.S.) had low levels of

serum IgG but normal levels of IgM and IgA. T-cell subpopulations in these patients were also different. Both had an increased proportion of Tγ cells but only one of them had a low proportion of Tμ cells.

Patients with common variable immunodeficiency usually have a normal or only slightly decreased proportion of circulating B lymphocytes associated with hypogammaglobulinaemia. T cells and third population cells are usually normal (Gupta, 1976; Gupta *et al.*, 1976b; Gajl-Peczalska *et al.*, 1973; 1973; Cooper *et al.*, 1972; Hayward & Greaves, 1975; Preud'homme *et al.*, 1975). This was also the case with the patients studied here. Six out of ten patients had normal proportions of Tμ and Tγ cells. Three had a low proportion of Tμ cells and two had an increased proportion of Tγ cells. Only one patient had both a low proportion of Tμ cells and an increased proportion of Tγ cells. Waldmann *et al.* (1976) and Siegal, Siegal & Good (1976) demonstrated that certain patients with common variable immunodeficiency have an increased suppressor T-cell activity. Nelson *et al.* (1976) reported that patients with common variable immunodeficiency associated with autoimmune diseases seem to lack increased suppressor cell activity. One of our patients (C.B.) who had hypogammaglobulinaemia associated with rheumatoid arthritis possessed normal proportions of Tγ cells.

Patients with selective IgA deficiency show a normal profile of lymphocyte subpopulations including cells with surface IgA (Schiff *et al.*, 1974; Gupta & Good, 1977). All the patients with selective IgA deficiency studied had normal proportions of Tμ and Tγ cells except one patient, who had an increased proportion of Tγ cells. Occasional patients with selective IgA deficiency have been shown to have an increased T cell suppressor activity (Waldmann *et al.*, 1976).

Steinberg *et al.* (1975) demonstrated loss of thymic suppressor function in NZB and NZW mice and suggested that this abnormality is pathogenetically related to the progressive development of autoimmunity and lymphoid hyperplasia. They also reported that the development of autoimmunity could be inhibited by reconstitution with suppressor cells. Krakauer, Waldmann & Strober (1976) also reported loss of suppressor T cells in adult NZB/NZW mice. The relationships, however, appear to be complex because at the time the autoimmunities become fully expressed and malignancy develops, increased spontaneous suppressor cell activity is demonstrable in the cells of the spleen in NZB mice (Roder, Bell & Singhal, 1975). Furthermore; decreased expression of autoimmunity, prevention of renal and vascular disease and prolongation of life by caloric restriction was associated with maintenance of immunological vigor and lack of activated suppressor cells in the spleen in NZB/NZW mice (Fernandes, Yunis & Good, 1976; Fernandes, Friend & Yunis, 1977). Recently, loss of suppressor T-cell function has also been reported in patients with systemic lupus erythematosus (Abdou *et al.*, 1976; Bresnihan, Jasin & Ziff, 1976). Our study demonstrates normal proportions of Tμ cells and low proportions of Tγ cells in two patients with this disease. A patient with essential mixed cryoglobulinaemia, however, did not demonstrate any alteration in the proportions of Tμ and Tγ cells. This finding in systemic lupus erythematosus must be greatly expanded by further study, but it represents a most provocative beginning.

The present study represents heterogeneity in the distribution of circulating Tμ and Tγ cells in patients with several immunodeficiencies. Our preliminary study of the Tμ and Tγ-cell proportions, together with *in vitro* assays of suppressor activity of T cells for immunoglobulin synthesis and secretion by B lymphocytes and terminally differentiated plasma cells in primary immunodeficiency and lymphoproliferative disorders, has revealed a good correlation between proportions of Tγ cells and *in vitro* suppressor activity (Gupta, Schwartz & Good, unpublished observations). From these observations, it is clear that the study of Tγ and Tμ cells may help to define the lymphocyte populations and functions in both immunodeficiency and autoimmune diseases. Furthermore, Cooper *et al.* (1976) have proposed that T helper and T suppressor relationships to the functions of these two interesting T-lymphocyte subclasses is pathogenetically important in primary immunodeficiency. Our findings would support continued pursuit of this working hypothesis.

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