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Enumeration of T lymphocytes and T lymphocyte subsets in autoimmune disease using monoclonal antibodies

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SUMMARY

The numbers of T lymphocytes, B lymphocytes, helper and suppressor T lymphocytes were measured in peripheral blood of patients with autoimmune disease (rheumatoid arthritis, erythema nodosum, Sjögren's disease, Wegener's disease, idiopathic thrombocytopenia, pernicious anaemia and Hashimoto's disease). B lymphocytes were enumerated by direct immunofluorescence and T lymphocytes by E rosette tests and by indirect immunofluorescence with OKT3.PAN. Helper and suppressor T lymphocytes were determined by indirect immunofluorescence with OKT4.IND and OKT8.SUP respectively. The numbers of T lymphocytes, B lymphocytes and helper T lymphocytes in patients with autoimmune disease were normal, but the numbers of suppressor T lymphocytes were significantly lower.

INTRODUCTION

Autoimmune diseases are characterized by aberrations in immunoregulation. This leads to B lymphocyte hyperactivity resulting in hypergammaglobulinaemia and the production of autoantibodies. It has been postulated that a lack of suppressor T cells with subsequent loss of modulating effects on B cells and T cells could lead to autoimmune disease. This concept has been substantiated in the NZB/NZW mouse model (Krakauer, Waldman & Strober, 1976; Steinberg *et al.*, 1977) and in SLE (Bresnihan & Jasin, 1977; Fauci *et al.*, 1978; Kaufman & Bostwick, 1979; Krakauer *et al.*, 1979; Morimoto, 1978; Morimoto, Abe & Homma, 1979).

Techniques for measuring suppressor T cell activity in peripheral blood of human beings are merely based on co-cultures of mononuclear cells (Bresnihan & Jasin, 1977; Fauci *et al.*, 1978; Kaufman & Bostwick, 1979; Krakauer *et al.*, 1979; Morimoto, 1978; Morimoto *et al.*, 1979). These techniques are difficult to standardize and require a specialized laboratory.

Recently, specific monoclonal antibodies (OKT) reactive with different antigenic determinants present on human T lymphocytes were developed by Kung *et al.* (1979). OKT antibodies were produced from mouse hybridomas derived according to the technique of Kohler & Milstein (1975). Among these antibodies, OKT3.PAN reacted with all peripheral T cells (Kung *et al.*, 1979), OKT4.IND could distinguish the inducer-helper subclass (Reinherz *et al.*, 1979a, 1979b), whereas OKT8.SUP reacted with the suppressor-cytotoxic subpopulation of T lymphocytes (Reinherz & Schlossman, 1980; Reinherz *et al.*, 1980a).

In the present study we measured the number of OKT3.PAN, OKT4.IND and OKT8.SUP positive cells in peripheral blood of healthy subjects and patients with autoimmune disease by means of indirect immunofluorescence.

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MATERIALS AND METHODS

Subjects. Twenty-one healthy adults aged between 22 and 59 years (mean 35 years) were used as donors of normal lymphocytes. Twenty-three patients with autoimmune disease were studied; there were eight males and 15 females aged between 14 and 73 years (mean 50 years). Fifteen suffered from classic or definite RA by ARA criteria. At the time of study their disease was evolutive and they had not received any basic anti-rheumatic therapy. The other patients were diagnosed as idiopathic thrombocytopenia (two), pernicious anaemia (one), Sjögren's disease (one), Wegener's disease (one), erythema nodosum (one) and Hashimoto's disease (two).

Separation of lymphocytes. Diluted blood was layered onto a Ficoll–Isopaque mixture and mononuclear cells (MNC) isolated as previously described (De Cock, De Cree & Verhaegen, 1980).

E rosette-forming cells (RFC). The E rosette formation of T lymphocytes with sheep red blood cells (SRBC) or SRBC treated with *S*-2-aminoethylisothiouronium bromide (RFC_{AET}) was determined as previously described (De Cock *et al.*, 1980; Kaplan & Clark, 1974).

OKT monoclonal antibodies. OKT3.PAN, OKT4.IND and OKT8.SUP monoclonal antibodies were kindly provided by Ortho Pharmaceutical Corporation, Raritan, New Jersey, USA. Stock solutions of the antibodies (100 μg/ml) were made in PBS containing 0·02% sodium azide, aliquoted and stored at −37°C.

Membrane immunofluorescence for OKT-positive cells. MNC (0·1 ml; 5×10^6/ml) were mixed with 0·1 ml buffer, OKT3.PAN (10 μg/ml), OKT4.IND (5 μg/ml) or OKT8.SUP (5 μg/ml) and incubated at 4°C for 30 min. After incubation the cells were washed three times in cold buffer and resuspended in 0·1 ml buffer. Cells were stained at 4°C for 30 min with an appropriate dilution of fluorescein-conjugated polyvalent anti-mouse immunoglobulin (Nordic, Leuven, Belgium). After staining, the cells were washed three times in cold PBS, 0·02% azide, and resuspended in one drop of the buffer. Tests were done in duplicate.

Membrane immunofluorescence was evaluated using a Leitz Orthoplan microscope equipped with I_2 (450–480 nm) excitation filter, a 515 barrier filter and a Ploem incident illuminator. One hundred lymphocyte-like cells were counted, monocytes being excluded from the counts by morphological criteria.

Membrane immunofluorescence for B cells. MNC (0·1 ml; 5×10^6/ml) were mixed with 0·1 ml buffer or 0·1 ml of an appropriate dilution of fluorescein-conjugated polyvalent anti-human immunoglobulin (Nordic, Leuven, Belgium) and incubated at 4°C for 30 min. After incubation cells were washed three times in cold buffer and resuspended in one drop of buffer. One hundred lymphocyte-like cells were counted, monocytes being excluded from the counts by morphological criteria.

RESULTS

The relative and absolute number of E rosette-forming cells (RFC, RFC_{AET}), inducer T cells (OKT4.IND) and B cells (sIg) in peripheral blood of patients with rheumatoid arthritis, erythema nodosum, Sjögren's disease, Wegener's disease, idiopathic thrombocytopenia, pernicious anaemia and Hashimoto's disease did not differ significantly from those in the blood of healthy subjects (Figs 1 and 2).

The relative number of OKT3.PAN positive lymphocytes, but not the absolute number, was significantly lower ($P \leqslant 0{\cdot}05$) in these patients with autoimmune disease than in healthy subjects.

The relative and absolute numbers of suppressor T cells (OKT8.SUP) were significantly lower in the patients with autoimmune disease than in healthy subjects ($P \leqslant 0{\cdot}01$ and $P \leqslant 0{\cdot}001$ respectively).

The ratio helper/suppressor cells was significantly higher ($P \leqslant 0{\cdot}001$) in the patients with autoimmune disease as compared to the ratio in healthy subjects (Fig. 3).

DISCUSSION

In a previous report we showed that the number of peripheral T lymphocytes in healthy subjects

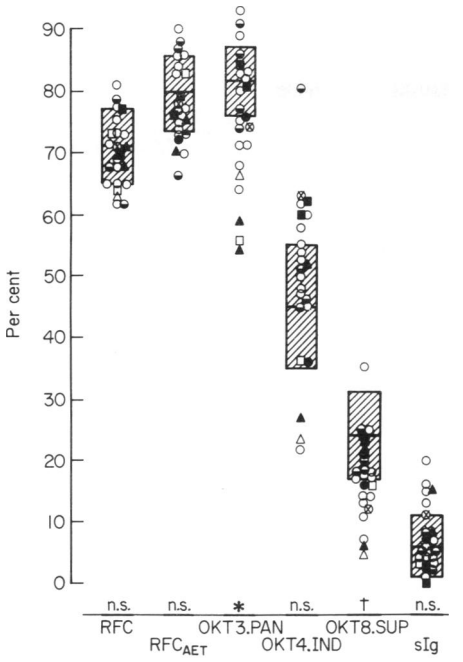

Fig. 1

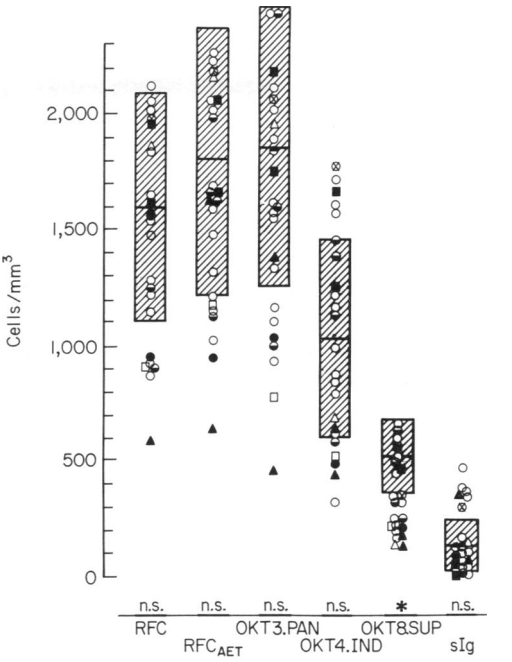

Fig. 2

Fig. 1. Relative numbers of E rosette-forming cells (RFC, RFC_{AET}), OKT3.PAN, OKT4.IND and OKT8.SUP positive cells and surface immunoglobulin-bearing cells (sIg) in peripheral blood of patients with autoimmune disease. The hatched area represents the mean of 21 healthy subjects ±sd. Statistical analysis, Student's *t*-test: n.s. = not significant, * $P \leqslant 0{\cdot}05$, † $P \leqslant 0{\cdot}01$. (○) Seropositive RA, (⊖) seronegative RA, (⊗) erythema nodosum, (●) Sjögren's diseases, (△) Wegener's disease, (▲) idiopathic thrombocytopenia, (□) pernicious anaemia, (■) Hashimoto's disease.

Fig. 2. Absolute numbers of E rosette-forming cells (RFC, RFC_{AET}), OKT3.PAN, OKT4.IND and OKT8.SUP positive cells and surface immunoglobulin-bearing cells (sIg) in peripheral blood of patients with autoimmune disease. The hatched area represents the mean of 21 healthy subjects ±s.d. Statistical analysis, Student's *t*-test: n.s. = not significant, § $P \leqslant 0{\cdot}001$. See legend to Fig. 1 for key.

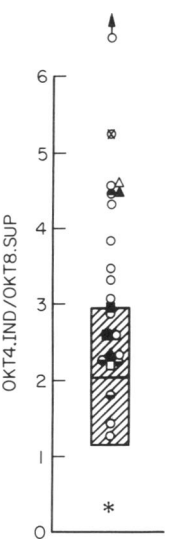

Fig. 3. The ratio helper/suppressor (OKT4.IND/OKT8.SUP) in peripheral blood of patients with autoimmune disease. The hatched area represents the mean of 21 healthy subjects ±s.d. Statistical analysis, Student's *t*-test: * $P \leqslant 0{\cdot}001$. See legend to Fig. 1 for key.

measured by OKT3.PAN monoclonal antibody correlated with the number of T lymphocytes measured by the enhanced E rosette test with AET-treated SRBC (De Cock *et al.*, 1980). In this study, OKT3.PAN monoclonal antibody detected a lower number of T lymphocytes in patients with autoimmune disease than the classic E rosette tests. Possible explanations could be: (a) a lower density of antigenic determinants for OKT3.PAN on T lymphocytes of patients with autoimmune disease; (b) serum factors released during active disease may hide OKT3.PAN determinants; and (c) disease-related metabolic changes in T lymphocytes of patients with autoimmune disease may repress OKT3.PAN determinant expression. However, the possible clinical relevance of diminished reactivity of OKT3.PAN monoclonal antibody with T lymphocytes of patients with autoimmune disease remains to be explored.

The human immune system consists of effector cells and regulatory T cells that are critical for immune homeostasis. These regulatory T cells can now be enumerated in human peripheral blood with monoclonal antibodies. OKT4.IND monoclonal antibody detects helper T cells (Reinherz *et al.*, 1979a, 1979b), whereas OKT8.SUP monoclonal antibody detects suppressor T cells (Reinherz *et al.*, 1980a). Major immunological abnormalities may result from alterations in the mature T cell subsets. In autoimmune disease lack of suppressor T cells with subsequent loss of modulating effects on B cells and T cells is thought to be responsible for the immunological abnormalities. Our results agree with this concept. The numbers of suppressor T cells (OKT8.SUP) were significantly lower in patients with autoimmune disease. The loss of suppressor T cells in autoimmune disease has also been reported for multiple sclerosis (Bach *et al.*, 1980) and autoimmune anaemia (Reinherz *et al.*, 1980b).

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