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Increased circulating Ia-positive T cells in patients with idiopathic thrombocytopenic purpura

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SUMMARY

Peripheral blood T cells from 40 patients with idiopathic thrombocytopenic purpura (ITP) were analysed for the presence of surface Ia antigens using monoclonal antibodies by indirect immunofluorescence. The percentage of Ia-positive (Ia+) T cells was significantly increased in patients with ITP ($6{\cdot}8 \pm 2{\cdot}9\%$, $P < 0{\cdot}005$) as compared with normal controls ($2{\cdot}3 \pm 0{\cdot}9\%$). There was an inverse correlation between the percentages of Ia+ T cells and platelet counts ($r = 0{\cdot}58$, $P < 0{\cdot}005$) and a positive correlation between the percentage of these cells and platelet-associated IgG (PAIgG) values ($r = 0{\cdot}55$, $P < 0{\cdot}01$). The percentage of Ia+ T cells was found to decrease within two weeks during therapy with high dose γ− globulin or corticosteroid. We have previously reported the presence of T cells bearing both helper/inducer (H/I) and suppressor/cytotoxic (S/C) phenotypes (double labelled cells, DLC) in patients with ITP and an inverse correlation between the percentages of DLC and their platelet counts. In the present study, we showed that a major part of Ia+ T cells had both H/I and S/C phenotypes. We also examined the correlation between Ia+ T cells and autologous mixed lymphocyte reaction (AMLR). A defective AMLR was demonstrated in patients with ITP. Furthermore, an inverse correlation was found between the percentages of Ia+ T cells and the proliferative responses to AMLR ($r = -0{\cdot}49$, $P < 0{\cdot}01$). These results suggest that increased circulating Ia+ T cells play a role in the abnormalities of the immunoregulatory system of ITP, especially in the regulation of autoantibody production.

Keywords idiopathic thrombocytopenic purpura Ia-positive T cell autologous mixed lymphocyte reaction

INTRODUCTION

Chronic idiopathic (immune) thrombocytopenic purpura (ITP) is a well defined autoimmune disorder caused by the binding of circulating antiplatelet antibodies to the patients' platelets, causing their destruction by the reticuloendothelial system (Karpatkin, 1980; McMillan, 1981). Although the existence of humoral antibodies in ITP has been well established, the mechanism of this antibody production, especially the functional abnormalities in T cell mediated-regulation, has remained only vaguely defined.

We have recently shown the presence of double-labelled cells (DLC) bearing both H/I and S/C phenotypes and an inverse correlation between the percentages of DLC and the platelet counts in

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ITP patients, and suggested that abnormalities in T cell subsets are related to the pathogenesis of ITP (Mizutani *et al.*, 1985).

Class II or I region-associated (Ia) antigens are a major histocompatibility complex encoded in surface glycoproteins which plays an important role in antigen presentation and in the regulation of immune response (Fu *et al.*, 1978; Ceuppens, Goodwins & Searles, 1981). In man, Ia antigens are expressed on the surface of B cells and monocytes (Winchester *et al.*, 1975). Recently it has become apparent that a small but definite number of normal peripheral blood T cells also express Ia antigens on their surface which play a major role in regulatory interactions between T and non-T cells, as reflected in the autologous mixed lymphocyte reaction (AMLR) (Katz *et al.*, 1975; Sauvezie *et al.*, 1982). Elevated levels of Ia-positive (Ia+) T cells have been reported in several diseases including some autoimmune disorders (Yu *et al.*, 1980; Jackson *et al.*, 1982; Pincus, Clegg & Ward, 1985; Koide, 1985; Zoumbos *et al.*, 1985; Ludgate *et al.*, 1981). However, there is no previous study on the incidence of Ia+ T cells in ITP. Here we have focused on circulating Ia+ T cells in ITP and investigated whether these cells correlate with disease activity. We also examined the correlation between Ia+ T cells and DLC or AMLR.

MATERIALS AND METHODS

Patients. Forty patients (mean age 39 years, range 15–72 years) with chronic ITP were studied. They all fulfilled the diagnostic criteria for chronic ITP previously described by Tsubakio *et al.* (1981); briefly, these are thrombocytopenia for over 6 months, absence of decreased megakaryocytes in the bone marrow, and exclusion of other underlying causes for thrombocytopenia such as SLE. None had clinical evidence of infection at the time of study and 10 patients had received some blood transfusion. Twenty-nine cases were receiving prednisolone, 5–30 mg/day, at the time of investigation. In five patients, daily intravenous injection of 0·4 g/kg intact γ-globulin ('Venoglobulin-I', Green Cross Corporation, Japan) was given for 5 days (Kurata *et al.*, 1983). In addition, 16 patients who fulfilled the revised American Rheumatism Association criteria for diagnosis for SLE (Tan *et al.*, 1982) and eight patients with aplastic anaemia (AA) were also investigated. Concurrent 20 controls were in good health and derived from laboratory personnel (mean age 31 years, range 23–47 years).

Cell isolation. Mononuclear cell (MNC) suspension was obtained from heparinized blood by Ficoll-Hypaque density gradient. MNC were washed three times in Hanks' balanced salt solution (HBSS) and suspended in RPMI 1640 (Flow Laboratories, North Ryde, NSW, Australia) supplemented with 10% fetal calf serum (FCS) (Flow Laboratories). Then, adherent cells were depleted by incubating MNC on plastic dishes for 60 min at 37°C in a moist atmosphere of 5% CO_2. T and B cells were separated from the non-adherent cells using sheep erythrocytes (SRBC) (Sakane, Steinberg & Green, 1978). These two fractions were washed three times in HBSS and resuspended in RPMI 1640 supplemented with 10% FCS. The isolated T cells were checked for contaminating B cells by staining with a monoclonal antibody (MoAb), BA-1 (Hybritec Incorporated, San Diego, California, USA). In all instances, 0·5–1·5% of the cells in the T cell preparation were BA-1 positive and more than 95% were SRBC-rosetted cells. The data were expressed by subtraction of the percentages of BA-1 positive cells in all cases. The viability of the recovered cells was always more than 90% as determined by trypan blue dye exclusion.

Monoclonal antibodies. OKIal (mouse IgG) was purchased from Ortho Pharmaceutical Corporation (Raritan, New Jersey, USA). To detect double marker expression of lymphocytes, cells were stained with the MoAb of different subclasses: BMA 040 (mouse IgG1 MoAb, H/I T) and BMA 081 (mouse IgG2 MoAb, S/C T). They were provided by Behring Institute (Marburg, FRG).

Indirect immunofluorescence and double staining experiments. Staining of lymphocytes with MoAb was performed using indirect immunofluorescence. Details of the methods have been described previously (Mizutani *et al.*, 1985).

Complement-mediated cytolysis. Isolated MNC, 1×10^6 in seven patients with ITP were incubated with 100 μl of RPMI 1640 containing 5 μl of OKIal for 30 min at 4°C. After washing, the cells were resuspended in RPMI 1640 containing the optimum concentration of rabbit complement

(Behring Institute). After incubation for 60 min at 37°C the cells were washed, then double staining (BMA 040 and BMA 081) was performed. After lysis, viable cells bearing Ia antigens were not detected.

Autologous mixed lymphocyte reaction (AMLR). Triplicate cultures were performed in round bottom microtitre plates (Nunc Inter Med. Denmark) in a volume of 200 μl containing 1×10^5 autologous non-T cells as stimulator cells. The stimulator cells were incubated with 50 μg/ml mitomycin C (Kyowa Hakko Co., Tokyo, Japan) at 37°C for 30 min and then washed three times in HBSS. The AMLR culture was incubated for 6 days in a 5% CO_2 incubator. To assess T cell proliferation, 0·5 μCi of ^{3}H-thymidine (Amersham International plc) was added to each well during the last 12 h of culture. The cultures were transferred to filter paper using automated cell harvester and thymidine incorporation was measured in a liquid scintillation counter. Data were expressed as the average of the counts per minute of triplicate cultures.

Platelet-associated IgG assay. For the evaluation of platelet-associated IgG (PAIgG), a micro enzyme-linked immunosorbant assay was used (Tsubakio *et al.*, 1981; 1983).

Statistical analysis. All data were analysed using Student's *t*-test.

RESULTS

Percentage of Ia+ T cells in patients with ITP and other disease. Figure 1 illustrates the percentages of Ia+ cells in T cell preparations defined by OKIa1 in patients with ITP and other diseases. The mean percentage of Ia+ T cells of 20 normal subjects was 2·3±0·9% (mean±s.d., range 0·5–4·5%), whereas that of 40 patients with ITP was increased to 6·8±2·9% (range 2·0–12·8%, $P<0·005$). In 16 patients with SLE, the percentages of these cells was 9·0±4·2% (range 2·0–14·0%, $P<0·001$). A slightly increased proportion of Ia+ T cells was also demonstrated in patients with aplastic anaemia, compared to normal controls (4·5±1·6%, range 2·5–7·0%, $P<0·05$).

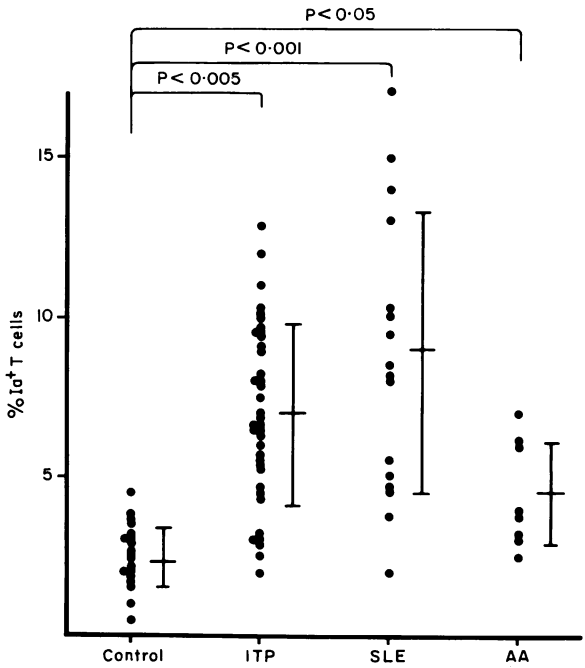

Fig. 1. Distribution of Ia+ T cells in T cell preparations in patients with ITP, SLE and AA. Ia+ T cells were detected using indirect immunofluorescence. Bars represent mean ±s.d.

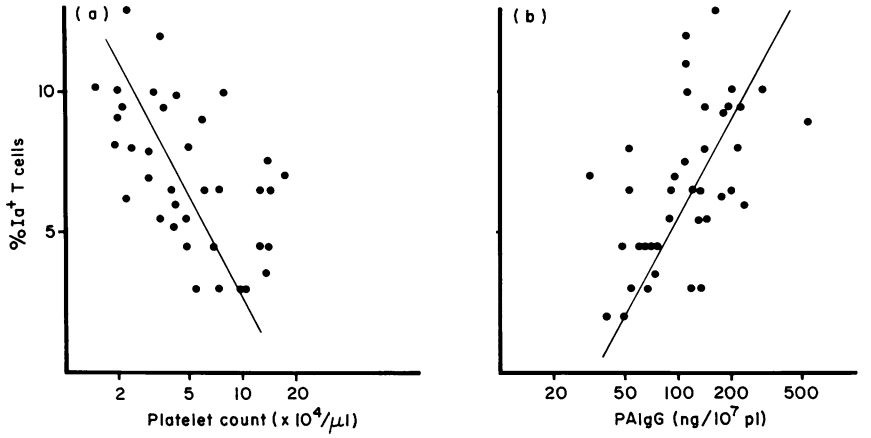

Fig. 2. Relationship between Ia+ T cells and (a) their platelet counts or (b) PAIgG values in patients with ITP. (a) $r=0{\cdot}58$, $P<0{\cdot}005$; (b) $r=0{\cdot}55$; $P<0{\cdot}01$).

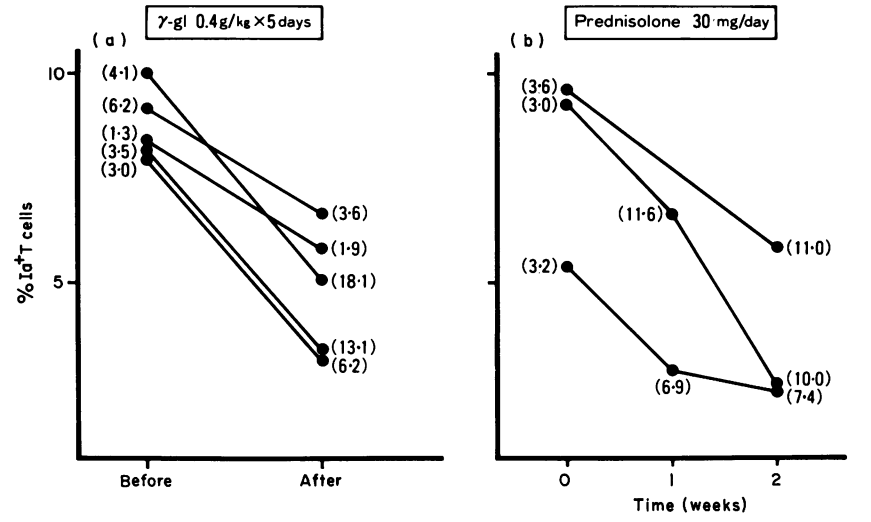

Fig. 3. Effect of (a) high dose γ-globulin or (b) corticosteroids on the percentages of Ia+ T cell in patients with ITP. The number in parentheses shows platelet count ($\times 10^4/\mu l$).

Correlation between Ia+ T cells and platelet counts or PAIgG values in patients with ITP. Figure 2 shows that there was an inverse correlation between the percentages of Ia+ T cells and the platelet counts ($r=-0{\cdot}58$, $P<0{\cdot}005$), and a positive correlation between the percentage of Ia+ T cells and the PAIgG values ($r=0{\cdot}55$, $P<0{\cdot}01$). It is obvious from this figure that the percentage of Ia+ T cells increased in parallel with the severity of the clinical state of disease.

Effect of therapy on the percentages of Ia+ T cell in patients with ITP. In five patients, the percentage of Ia+ T cells was studied before and on the 1st or 2nd day after the end of high dose γ-globulin therapy. As shown in Fig. 3, the percentage of Ia+ T cells decreased significantly during therapy (before: $8{\cdot}5\pm1{\cdot}0\%$, range 6·8–10·0%; after: $4{\cdot}5\pm2{\cdot}0\%$, range 3·0–7·2%) in all cases. An increase in the platelet count during therapy was found in all cases except two. The effect of corticosteroid therapy on the Ia+ T cells in three patients with ITP was also shown in Fig. 3. All three patients had not received any treatment previously. The percentage of these cells decreased to normal within 2 weeks of therapy (30 mg/day of prednisolone).

Table 1. Percentage of double labelled cells before and after lysis of Ia$^+$ T cells with OKIa 1 and complement

	% of DLC		
Patient	None	OKIa1 + C′	C′ only
1	5·2	1·8	6·2
2	7·1	2·0	5·5
3	8·7	1·2	9·0
4	8·8	1·2	9·0
5	9·0	4·9	7·4
6	6·2	4·0	8·2
7	7·9	2·1	6·0
Mean ± s.d.	7·7 ± 2·0	2·9 ± 1·5	7·0 ± 1·9

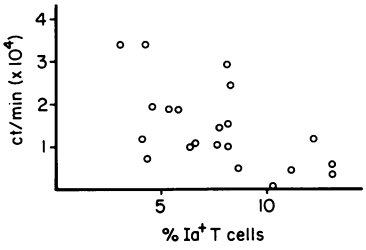

Fig. 4. Correlation between ^{3}H-thymidine incorporation in AMLR and the percentages of Ia + T cells in 20 patients with ITP. $r = -0{\cdot}49$, $P < 0{\cdot}01$.

Complement-mediated cytotoxicity assay. To evaluate the relationship between Ia + T cells and DLC which express both H/I and S/C phenotypes, we examined the percentages of DLC before and after killing of Ia + T cells with OKIa1 and rabbit complement. In seven patients with ITP, percentage of DLC was decreased after killing of Ia + T cells, showing that a major part of Ia + T cells have both H/I and S/C phenotypes (Table 1).

Autologous mixed lymphocyte reaction. The mean value for the AMLR in 10 normal controls was 30,112 ± 6,922 ct/min. In contrast, that of 20 patients with ITP was 13,554 ± 7,029 ct/min. There was a significant difference between the two ($P < 0{\cdot}001$). Furthermore, in these patients, there was an inverse correlation between the proliferative response to AMLR and the percentage of circulating Ia + T cells ($r = -0{\cdot}49$, $P < 0{\cdot}01$) (Fig. 4).

DISCUSSION

Our study showed that circulating Ia + T cells in patients with ITP were significantly increased compared with normal controls. The increase of Ia + T cells in ITP was not as prominent as in SLE. Our data in SLE were similar to those of other investigators (Yu *et al.*, 1980). It has been reported that Ia antigen is expressed on activated T cells stimulated by mitogens, alloantigens, or foreign antigens (Evans *et al.*, 1978; Ko *et al.*, 1979). Although about one-third of the patients studied had been transfused previously, no significant difference of the percentage of Ia + T cells was observed between the transfused and non-transfused groups. Furthermore, the percentage of these cells in patients with aplastic anaemia which had been receiving multiple transfusions was much lower than

in ITP patients. We have previously found an increase in circulating T cells bearing 'activated' antigens detected with OKT10 (Mizutani *et al.*, 1985), which seems to be compatible with the present data. These results indicate that Ia+ T cells in ITP patients were not stimulated by alloantigens but already activated *in vivo*.

Since it is important to determine whether Ia+ T cells found in the peripheral blood of patients with ITP are causally related to the disease process, we compared the correlation between the percentage of Ia+ T cells and disease activity, and analysed the changes during treatment. We have found a good correlation between the percentage of Ia+ T cells and disease activity and the decrease of Ia+ T cells during corticosteroid or high dose γ-globulin therapy. We have also previously demonstrated the suppressive effect of γ-globulin on the antibody production with alteration of T cell subsets (Tsubakio *et al.*, 1983). These results suggest that abnormalities of T cell subsets, including Ia+ T cells, are closely related to immunological abnormalities present in ITP, especially to the regulation of antibody production.

In a previous report, we have shown a significant increase of DLC in ITP and a correlation between peripheral platelet count and DLC (Mizutani *et al.*, 1985). In this study, we showed a significant portion of Ia+ T cells in ITP express both H/I and S/C phenotypes. An increase of circulating DLC has been also reported in some autoimmune diseases such as myasthenia gravis, rheumatoid arthritis (RA), chronic active hepatitis and Behçet disease (Berrih *et al.*, 1981; Pincus *et al.*, 1985; Valesini *et al.*, 1985). On the other hand, Blue *et al.* (1984) detected the presence of a small number of DLC (about 3%) in normal healthy donors and showed that lectins were able to increase the coexpression of T4 and T8 on a fraction of peripheral blood T cells. Our previous study found that DLC bore no detectable amounts of T6, an antigen found on the majority of T4+T8+ thymocytes despite expression of T10 antigen. We therefore speculated that DLC present in ITP patients might be prematurely released from the thymus at a later stage of intrathymic T cell differentiation. However, the present study and data from studies *in vitro* by Blue *et al.* (1984) suggest that some T cell activation in peripheral blood results in the generation of DLC population which is distinct from immature thymocytes. We have also shown a defective AMLR in ITP patients, a finding which was similar to that of Zinberg *et al.* (1982) and have found an inverse correlation between the percentage of Ia+ T cells and the AMLR response. Depressed AMLR have been generally observed in various autoimmune diseases, especially in SLE and RA (Sakane *et al.*, 1978; Gupta, 1983). On the other hand, Innes *et al.* (1979) reported that activity of suppressor cells was generated during AMLR and that suppressor cells generated in AMLR suppress antibody production *in vitro*. Sakane, Sternberg & Green (1980) and Smith & Dehoratius (1982) reported that defective AMLR in SLE with active phase returned to normal when the disease became inactive. In the defective AMLR, one of the mechanisms for protection against autoimmunity fails resulting in autoimmune disease. Furthermore, an inverse correlation between the defective AMLR and the percentage of Ia+ T cells has been also demonstrated in chronic active hepatitis and Sjögren's syndrome (Fukui *et al.*, 1984; Sauvezie *et al.*, 1982). Thus in ITP, a defective AMLR involving the increase of Ia+ T cells might play an important role in the production of autoantibodies against platelet.

In conclusion, the expression of Ia antigen on T cells may be closely related to the pathogenesis of autoimmunity in ITP. These findings may be important for evaluating the immunoregulatory function in ITP.

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REFERENCES

BERRIH, S., GAUD, C., BACH, M.A., BRICAND, H., BINET, J.P. & BACH, J.F. (1981) Evaluation of T cell subsets in myasthenia gravis using anti-T cell monoclonal antibodies. *Clin. exp. Immunol.* **45,** 1.

BLUE, M.L., DALEY, J.F., LEVINE, H. & SCHLOSSMAN, S.F. (1985) Coexpression of T4 and T8 on peripheral blood T cells demonstrated by two color fluorescence flow cytometry. *J. Immunol.* **134,** 2281.

CEUPPENS, J.L., GOODWIN, J.S. & SEARLES, R.P. (1981) The presence of Ia antigen on human peripheral blood T cells and T-cell subsets: analysis with monoclonal antibodies and the fluorescence-activated cell sorter. *Cell. Immunol.* **64,** 277.

EVANS, R.L., FALDETTA, T.J., HUMPHREY, R.E., PRATT, D.M., YUNIS, E.J. & SCHLOSSMAN, S.F. (1978) Peripheral human T cells sensitized in mixed leukocyte culture synthesize and express Ia-like antigens. *J. exp. Med.* **148,** 1440.

FU, S.M., CHIORAZZI, N., WANG, C.Y., MONTAZERI, G., KUNKEL, H.G., KO, H.S. & GOTTLIEB, A.B. (1978) Ia-bearing T lymphocytes in man: their identification and role in the generation of allogenic helper. *J. exp. Med.* **148,** 1423.

FUKUI, K., KAKUMU S., MURAKAMI, S., KURIKI, J., YOSHIOKA, K. & SAKAMOTO, N. (1984) Increased peripheral blood Ia positive T cells and their effect on autologous mixed lymphocyte reaction in chronic active liver disease. *Clin. exp. Immunol.* **58,** 90.

GUPTA, S. (1983) Autologous mixed lymphocyte reaction in health and disease states in man. *Vox Sang.* **44,** 265.

INNES, J.B., KUNTZ, M.M., TAI KING, Y. & WEKSLER, M.E. (1979) Induction of suppressor activity in the autologous mixed lymphocyte reaction and in cultures with concanavalin A. *J. clin. Invest.* **64,** 1608.

JACKSON, R.A., MORRIS, M.A., HAYNES, B.F. & EISENBARTH, G.S. (1982) Increased circulating Ia-antigen-bearing T cells in type I diabetes mellitus. *N. Engl. J. Med.* **306,** 785.

KARPATKIN, S. (1980) Autoimmune thrombocytopenic purpura. *Blood* **56,** 329.

KATZ, D.H., GRAVES, M., DORF, M.E., DIMUZIO, H. & BENACERRAF, B. (1975) Cell interactions between histocompatible T and B lymphocytes. Cooperative responses between lymphocytes and controlled by genes in the I region of H-2 complex. *J. exp. Med.* **141,** 263.

KO, H.S., FU, S.M., WINCHESTER, R.J., YU, D.T.Y. & KUNKEL, H.G. (1979) Ia determinations on stimulated T cells. *J. exp. Med.* **150,** 246.

KOIDE, J. (1985) Functional property of Ia-positive T cell in peripheral blood from patients with systemic lupus erythematosus. *Scand. J. Immunol.* **22,** 557.

KURATA, Y., TSUBAKIO, T., YONEZAWA, T. & TARUI, S. (1983) High-dose gammaglobulin therapy for idiopathic thrombocytopenic purpura in adults. *Acta Haematol.* **69,** 391.

LUDGATE, M.E., MCGREGOR, A.M., WEETMAN, A.P., RATANACHAIYAVONG, S., LAZARUS, J.H., HALL, R. & MIDDLETON, G.W. Analysis of T cell subsets in Graves' disease: alterations associated with carbimazole. *Br. med. J.* **288,** 526.

MCMILLAN, R. (1981) Chronic idiopathic thrombocytopenic purpura. *N. Engl. J. Med.* **304,** 1135.

MIZUTANI, H., KATAGIRI, S., UEJIMA, K., OHNISHI, M., TAMAKI, T., KANAYAMA, Y., TSUBAKIO, T., KURATA, Y., YONEZAWA, T. & TARUI, S. (1985) T-cell abnormalities in patients with idiopathic thrombocytopenic purpura: the presence of OKT4+8+ cells. *Scand. J. Haematol.* **35,** 233.

PINCUS, S.H., CLEGG, D.O. & WARD, J.R. (1985) Characterization of T cells bearing HLA-DR antigens in rheumatoid arthritis. *Arthritis. Rheum.* **28,** 8.

SAKANE, T., STEINBERG, A.D. & GREEN, I. (1978) Failure of autologous mixed lymphocyte reaction between T and non-T cells in patients with systemic lupus erythematosus. *Proc. natn Acad. Sci. USA.* **75,** 3464.

SAKANE, T., STEINBERG, A.D. & GREEN, I. (1980) Studies of immune functions of patients with systemic lupus erythematosus. V. T cell suppressor function and autologous mixed lymphocyte reaction during active and inactive phase of disease. *Arthritis. Rheum.* **23,** 225.

SAUVEZIE, B., MIYASAKA, N., CHARRON, D., KIELICH, C., LOEB, J., DANIELS, T.E. & TALAL, N. (1982) An increase in peripheral blood Ia-positive T cells in Sjögren's syndrome correlates with a decrease in the autologous mixed lymphocyte response. *Clin. exp. Immunol.* **49,** 50.

SMITH, J.B. & DEHORATIUS, R.J. (1982) Different autologous mixed lymphocyte reactions correlate with disease activity in systemic lupus erythematosus and rheumatoid arthritis. *Clin. exp. Immunol.* **48,** 155.

TAN, E.M., COHEN, A.S., FRIES, J.F., MASI, A.T., MCSHANE, D.J., ROTHFIELD, N.F., SCHALLER, J.G., TALAL, N. & WINCHESTER, F.J. (1982) The 1982 revised criteria for the classification of systemic lupus erythematosus. *Arthritis Rheum.* **25,** 1271.

TSUBAKIO, T., KURATA, Y., YONEZAWA, T. & KITANI, T. (1981) Quantification of platelet-associated IgG with competitive solid-phase enzyme immunoassay. *Acta Haematol.* **66,** 251.

TSUBAKIO, T., KURATA, Y., KATAGIRI, S., KANAKURA, Y., TAMAKI, T., KUYAMA, J., KANAYAMA, Y., YONEZAWA, T. & TARUI, S. (1983) Alteration of T cell subsets and immunoglobulin synthesis *in vitro* during high dose γ-globulin therapy in patients with idiopathic thrombocytopenic purpura. *Clin. exp. Immunol.* **53,** 697.

VALESINI, G., PIVETTI-PEZZI, P., MASTRANDREA, F., MONCADA, A., UOMO, M. & NATARI, P.G. (1985) Evaluation of T cell subsets in Behçet's syndrome using anti-T cell monoclonal antibodies. *Clin. exp. Immunol.* **60,** 55.

WINCHESTER, R.J., FU, S.M., WERNET, P., KUNKEL, H.G., DUPONT, B. & JERSID, C. (1975) Recognition by pregnancy serums of non-HLA alloantigens selectively expressed on B lymphocytes. *J. exp. Med.* **141,** 924.

YU, D.T.Y., WINCHESTER, J., FU, S.M., GIBOFSKY, A., KO, H.S. & KUKEL, H.G. (1980) Peripheral blood Ia-positive T cells: increase in certain disease and after immunization. *J. exp. Med.* **151,** 91.

ZINBERG, M., FRANCUS, T., WEKSLER, M.E., SISKIND, G.W. & KARPATKIN, S. (1982) Abnormal autologous mixed lymphocyte reaction in autoimmune thrombocytopenic purpura. *Blood.* **59,** 148.

ZOUMBOS, N.C., GASCON, P., DJEU, J.Y., TROST, S.R. & YOUNG, N.S. (1985) Circulating activated suppressor T lymphocytes in aplastic anemia. *N. Engl. J. Med.* **312,** 257.