T-Cell Subsets Analyzed by Monoclonal Antibodies in Patients with Primary Immunodeficiency Diseases

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—The proportion of T-cell subsets was normal in all patients with X-linked agammaglobulinemia except for an 8-month patient who showed a decrease in OKT8⁺ T-cell population. In two patients with hypogammaglobulinemia with IgM production, T-cell subsets were normal in distribution and the patients' B cells produced only IgM in culture with autologous T cells. A patient with common variable immunodeficiency showed no imbalance in distribution of T-cell subsets. Imbalance of regulatory T-cell subsets was found in a patient with DiGeorge syndrome and severe combined immunodeficiency. ——— T-cell subsets; immunodeficiency disease; agammaglobulinemia; immunoglobulin production

Immunoglobulin production is under the control of regulatory T-cell subpopulations (Cantor and Boyse 1977). Recently, T-cell subsets, functionally distinct, have been identified by monoclonal antibodies that selectively react to those populations (Reinherz et al. 1979a, b, c). There have been several reports of abnormalities in distribution of T-cell subsets in patients with primary immunodeficiency, suggesting their important role in the pathogenesis of immunodeficiencies. The present communication describes a study on T-cell subsets defined by monoclonal antibodies, combined with in vitro functional assays of the lymphocytes in patients with a variety of primary immunodeficiency diseases.

MATERIALS AND METHODS

Patients

Ten patients with various types of primary immunodeficiency diseases were studied. Four male patients including two siblings had X-linked agammaglobulinemia, three female patients common variable immunodeficiency, one male patient DiGeorge syndrome, and two male patients severe combined immunodeficiency (SCID), one of which exhibited a selective T cell defect (Tsuchiya et al. 1983a). Diagnosis was made on the basis of criteria reported

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TABLE 1. *T-cell subsets in patients*

Patients	Age (years)	Sex	Lymphocyte count (/mm ³)	E _N (%)	sIg M (%)
1 M.O.	4	M	3,300	92	0
2 O.S.	12	M	1,790	91	0
3 K.Ko.	6	M	5,400	91	0
4 K.Y.	8/12	M	6,400	90	0
5 C.J.	7	F	3,150	77	4.5
6 K.Ky.	17	F	2,970	84	2.4
7 O.H.	12	F	3,070	76	6.2
8 W.G.	4	M	5,700	26	11.5
9 Y.Ya.	4/12	M	2,890	59	2.9
10 Y.A.	8/12	M	4,680	3	59
Controls (mean $\pm$ s.d.)				70.0 $\pm$ 7.2	4.8 $\pm$ 1.3

Patients 1-4, X-linked agammaglobulinemia; Patients 5-7, common variable combined immunodeficiency; E_N, neuraminidase treated sheep erythrocytes; sIg M, cells; ND, not done.

previously (Report of a WHO Scientific Group 1978). At the time of investigation, all patients except for one with DiGeorge syndrome were undergoing γ -globulin-replacement therapy.

Lymphocytes isolation and surface markers

Peripheral blood mononuclear cells were isolated from heparinized venous blood of patients by Ficoll-Paque (Pharmacia, Uppsala, Sweden) density gradient centrifugation (Böyum 1968). Sheep erythrocyte rosette forming cells (E_N⁺ cells) and membrane immunoglobulin bearing lymphocytes (sIg⁺ cells) were identified by the method described previously (Tsuchiya et al. 1980).

Lymphocyte proliferation with mitogens

The proliferative responses to phytohemagglutinin (PHA), concanavalin A (Con A), and pokeweed mitogen (PWM) were measured in accordance with the methods described previously (Tsuchiya et al. 1980).

Monoclonal antibody analysis of lymphocytes by indirect immunofluorescence

For analysis of T-cell subsets, four monoclonal antibodies, OKT3, OKT4, OKT6, and OKT8 (Kung et al. 1979; Reinherz et al. 1980) were used. Monoclonal antibodies, including ones reacting selectively to B cell specific antigen (B1) (Stashenko et al. 1980) and to Ia-like antigen, were purchased from Coulter Clone TM Co. Hialeah, Florida. An indirect immunofluorescence assay was used to enumerate peripheral blood T-cell subsets, B1 positive, and Ia-like antigen positive cells. Peripheral blood mononuclear cells isolated by Ficoll-Paque density gradient centrifugation were passed through a Sephadex G-10 (Pharmacia, Uppsala, Sweden) column to remove macrophages and other large adherent cells causing non-specific fluorescence and mixed with an optimal concentration of monoclonal antibodies. Fluorescein isothiocyanate-conjugated F(ab')₂ fragment goat anti-mouse IgG (G/M FITC) (Cappel Laboratories, Cochaville, Pa. 19330) were used as a second antibody. Assays with monoclonal antibodies were performed more than twice at different times for individuals.

with primary immunodeficiency diseases

Ia-like (%)	B 1 (%)	OKT3 (%)	OKT4 (%)	OKT6 (%)	OKT8 (%)	OKT4 OKT8
4	0	94	65	0	27	2.4
6	0	93	62	0	36	1.7
5	1	94	65	0	32	2.0
3	0	95	79	0	19	4.0
12	6	89	62	0	40	1.7
13	9	94	62	0	40	1.5
16	13	89	57	0	36	1.6
7	ND	80	59	0	18	3.2
ND	ND	52	1	0	53	0.02
77	63	5	1	0	0	—
18.2±3.2	9.1±2.0	68.3±10.9	41.8±11.5	0	27.4±2.9	1.5±0.3

immunodeficiency; Patient 8, DiGeorge syndrome (partial); Patients 9 & 10, severe Ia-like, B 1, OKT3, 4, 6, 8, the percentages of those of peripheral blood mononuclear

In vitro immunoglobulin production stimulated by PWM

Helper function of lymphocytes in Ig synthesis was investigated in PWM assay, as described elsewhere (Lipsky et al. 1978; Tsuchiya et al. 1981). In brief, T cell-enriched fractions were obtained from mononuclear cells by density sedimentation of spontaneous sheep red blood cell (SRBC)-rosetting using SRBC pretreated with neuraminidase (Behringwerke, Marburg, W. Germany) (Weiner et al. 1973; Siegal et al. 1978). Non-rosetting mononuclear cells were termed B cell-enriched fraction. All cultures were incubated at 37° C with 5% CO₂ in air for 7 days. Immunoglobulins secreted in culture supernatants were measured by inhibition radioimmunoassay as described previously (Platts-Mills and Ishizaka 1975; Tsuchiya et al. 1980).

RESULTS

The distribution of T-cell subsets defined by monoclonal antibodies and several other surface markers of peripheral lymphocytes from patients examined is shown in Table 1. In patients with X-linked agammaglobulinemia, the majority of peripheral lymphocytes were OKT3⁺ cells, corresponding to the population of E_N⁺ cells. Three patients (Nos. 1, 2, 3) had a normal ratio of OKT4⁺ inducer and OKT8⁺ suppressor cells. One patient (No. 4) at age 8 months when examined had an increase in the OKT4/OKT8 ratio.

In three patients with common variable immunodeficiency with a circulating B cell population an increase in the proportion of OKT3⁺ cells was observed. These cells contained an excess of OKT8⁺ suppressor cells as judged by a decreased ratio of OKT4⁺ and OKT8⁺ cells.

A patient with congenital thymic aplasia (DiGeorge syndrome) had a normal number of OKT3⁺ cells combined with a low proportion of E_N⁺ cells in peripheral lymphocytes. The ratio of OKT4⁺ and OKT8⁺ cells was found to be higher than

that in controls.

In severe combined immunodeficiency, one patient (No. 9) had a selective OKT4⁺ cell deficiency. The proportion of OKT3⁺ cells was 52% and that of OKT8⁺ cells 53%. In contrast, OKT4⁺ cells were found only rarely by the immunofluorescence technique, and comprised only one percent. Another patient (No. 10) with SCID and a few circulating cells bearing mature T-cell antigens. There were no circulating cells with the immature T-cell antigen OKT6. How-

Fig. 1. In vitro immunoglobulin production in patients with X-linked agammaglobulinemia. Autologous and allogeneic T-B mixtures were cultured in the presence of PWM for 7 days. IgM (open columns) and IgG (dotted columns) secretions were measured by an inhibition radioimmunoassay. Bp and Tp represent B cell- and T cell-enriched fractions from control Bn and Tn, B cell- and T cell-enriched fractions from control subjects and TpM and TnM, T cell-enriched fractions treated with mitomycin C. A: Patient M.O. B: Patient K.Ko. C: Patient O.S. D: Patient K.Y.

Fig. 2. In vitro immunoglobulin production in patients with hypogammaglobulinemia with IgM production. Procedures and abbreviations are the same as those for Fig. 1. A: Patient C.J. B: Patient K.Ky.

ever, this patient had cells bearing the C3b complement receptor (98%) and B1 antigen (63%).

To determine whether there were functional defects in the OKT4⁺ inducer population, the effect of T cells from patients with X-linked agammaglobulinemia and common variable immunodeficiency on in vitro immunoglobulin production by their own B cells and allogeneic B cells was examined. As shown in Figs. 1 and 2, T cells from four patients with X-linked agammaglobulinemia failed to induce immunoglobulin production by normal B cells, but their mitomycin C-treated T cells induced immunoglobulin production by normal B cells as did T cells derived from normal adults. On the other hand, non-E_N rosetting cells defined as "B cells" from patients with X-linked agammaglobulinemia secreted none of the immunoglobulins in any of the culture systems tested. In patients with common variable immunodeficiency, their mitomycin C-treated T cells induced IgM production in coculture with autologous and allogeneic B cells.

DISCUSSION

In the pretest study, lymphocytes from patients with a variety of primary immunodeficiency diseases were analyzed using a series of monoclonal antibodies. Most patients with X-linked agammaglobulinemia had a normal population of the two major subsets of peripheral T cells, OKT4⁺ helper/inducer cells and reciprocal OKT8⁺ suppressor/cytotoxic cells. The absence of a predominant T cell subset deficiency in those patients confirms the view that the disease is caused by a defect in B cell differentiation. In fact, patients with X-linked agammaglobulinemia have pre-B cells in the bone marrow but lack mature B cells to produce immunoglobulins (Cooper and Seligmann 1977). On the other hand, an imbalance of regulatory T-cell subsets has been observed in some patients with the disease (Reinherz et al. 1981), allowing the assumption that pre-B cells are unable to mature into B lymphocytes due to a block exerted by regulatory T cells (Aiuti et al. 1982). However, the T-cell subset abnormalities appear to be secondary to the B cell defects themselves or to be induced by the gammaglobulin therapy (Reinherz et al. 1981). In this regard, while most patients with X-linked agammaglobulinemia had normal T-cell subset distribution, the youngest child showed an increase in the ratio of OKT4⁺ inducer and OKT8⁺ suppressor cells. However, such increase in the OKT4⁺/OKT8⁺ ratio is commonly found in normal young children (Phan-Dinh-Tuy et al. 1981). Agammaglobulinemia patients examined here have been shown to have precursor B cells in the bone marrow (Tsuchiya et al. 1983b), suggesting an aberrant differentiation along cells of B lineage. Normal T-cell subset patterns and spontaneous helper activities in PWM-induced immunoglobulin synthesis may support the view of B cell defects in the pathogenesis of X-linked agammaglobulinemia.

Common variable immunodeficiency, though being heterogenous in nature, is characterized by hypogammaglobulinemia with immunoglobulin-bearing B cells

(Report of a WHO Scientific Group 1978). While defects in B cells are considered to be the primary cause in many cases of the disease (Sakai et al. 1982), T-cell dysfunctions are often found, indicating their involvement in the pathogenesis of the disease (Waldmann et al. 1974; Moretta et al. 1977; Gupta and Good 1978). In this regard, the OKT4⁺ inducer cells enhance immunoglobulin production whereas the OKT5⁺/OKT8⁺ suppressor subset can suppress immunoglobulin production by B cells. The ratio of OKT4⁺ and OKT5⁺/OKT8⁺ cells is known to be related to the degree of suppression (Reinherz et al. 1980). The imbalance of OKT4⁺ and OKT8⁺ observed in our patients with common variable immunodeficiency is in accord with other's observation (Aiuti et al. 1982), supporting the view that an excess of suppressor activity plays an important role in the pathogenesis of common variable immunodeficiency. In accordance with the imbalance of T-cell subsets, mitomycin C-treated lymphocytes from patients with varied immunodeficiencies exert a helper function in PWM-stimulated immunoglobulin production systems, as well as those from normal controls.

In DiGeorge syndrome the presence of mature T cells indicates that the patients have a small thymic rudiment (Lischner 1972). A decrease in the percentage of OKT8⁺ suppressor cells in relation to OKT4⁺ inducer cells (the high OKT4⁺ : OKT8⁺ ratio) found in our patient is consistent with other researchers' observations (Reinherz et al. 1981). However, there was a dissociation between the percentages of OKT3⁺ mature T cells and E_N⁺ T cells. Such an aberrant distribution of mature T cells was seen in one of 5 patients with DiGeorge syndrome reported by Reinherz et al. However, the significance of this phenomenon is unknown.

Severe combined immunodeficiency comprised a heterogeneous group of diseases with T and B cell differentiation defects. One of the patients examined has lacked any peripheral lymphocytes with mature T cell antigens, meeting the criteria for the first or second group of severe combined immunodeficiency defined by monoclonal antibodies (Reinherz et al. 1981). Another patient with selective OKT4⁺ T-cell deficiency was described in detail elsewhere (Tsuchiya et al. 1983a). The patient's lymphocytes had neither helper function nor suppressor activity in PWM-induced immunoglobulin production in spite of the presence of OKT8⁺ cells.

In summary, analysis of T-cell subsets by monoclonal antibodies combined with *in vitro* studies of their function provides some suggestions and questions about the pathogenesis of immunodeficiencies. An imbalance of regulatory T-cell subsets is involved not only in the pathogenesis of diseases with the impairment of cellular immunity but also in those with humoral impairment.

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References

- 1) Aiuti, F., Pandolfi, F., Fiorilli, M., Bonomo, R., Quinti, I., Frielingsdorf, A. & Luzi, G. (1982) Monoclonal antibody analysis of T cell subsets in 40 patients with immunodeficiencies. *J. clin. Immunol.*, **2**, 81-89.
- 2) Böyum, A. (1968) Isolation of mononuclear cells and granulocytes from human blood. *Scand. J. clin. Lab. Invest.*, Supple. **97**, 1-76.
- 3) Cantor, H. & Boyse, E.A. (1977) Regulation of cellular and humoral immune responses by T cell subclasses. *Cold Spring Harbour Symp. Quant. Biol.*, **41**, 23-32.
- 4) Cooper, M.D. & Seligmann, M. (1977) B and T lymphocytes in immunodeficiency and lymphoproliferative diseases. In: *B and T Cell in Immune Recognition*, edited by F. Loor & G.E. Roelants, John Wiley & Sons, London, pp. 378-405.
- 5) Gupta, S. & Good, R.A. (1978) Subpopulations of human T lymphocytes. V. T lymphocytes with receptors for immunoglobulin M or G in patients with primary immunodeficiency disorders. *Clin. Immunol. Immunopath.*, **11**, 292-301.
- 6) Kung, P.C., Goldstein, G., Reinherz, E.L. & Schlossman, S.F. (1979) Monoclonal antibodies defining distinctive human T cell surface antigens. *Science*, **206**, 347.
- 7) Lipsky, P.E., Ginsburt, W.W., Finkelman, F.D. & Ziff, M. (1978) Control of human B lymphocyte responsiveness: Enhanced suppressor T cell activity after *in vitro* incubation. *J. Immunol.*, **120**, 902-910.
- 8) Lischer, H.W. (1972) DiGeorge syndrome(s). *J. Pediatr.*, **81**, 1042-1044.
- 9) Moretta, L., Mingari, M.C., Webb, S.R., Pearl, E.R., Lydyard, P.M., Grossi, C.E., Lawton, A.R. & Cooper, M.D. (1977) Imbalances in T cell subpopulations associated with immunodeficiency and autoimmune syndromes. *Europ. J. Immunol.*, **7**, 696-700.
- 10) Phan-Dinh-Tuy, F., Darandy, A., Griscelli, C. & Buch, M.A. (1981) T cell subset analysis by monoclonal antibodies in primary immunodeficiencies. *Scand. J. Immunol.*, **14**, 193-200.
- 11) Platts-Mills, T.A.E. & Ishizaka, K. (1975) IgG and IgA diphtheria antitoxin responses from human tonsil lymphocytes. *J. Immunol.*, **114**, 1058-1064.
- 12) Reinherz, E.L., Kung, P.C., Goldstein, G. & Schlossman, S.F. (1979a) A monoclonal antibody with selective reactivity with functionally mature thymocytes and all peripheral human T cells. *J. Immunol.*, **123**, 1312-1317.
- 13) Reinherz, E.L., Kung, P.C., Goldstein, G. & Schlossman, S.F. (1979b) Further characterization of the human inducer T cell subset defined by monoclonal antibody. *J. Immunol.*, **123**, 2884-2896.
- 14) Reinherz, E.L., Kung, P.C., Goldstein, G. & Schlossman, S.F. (1979c) Separation of functional subsets of human T cells by a monoclonal antibody. *Proc. nat. Acad. Sci. USA*, **76**, 4061-4065.
- 15) Reinherz, E.L., Kung, P.C., Goldstein, G., Levey, R.H. & Schlossman, S.F. (1980) Discrete stages of human intrathymic differentiation. Analysis of normal thymocytes and leukemic lymphoblasts of T cell lineage. *Proc. nat. Acad. Sci. USA*, **77**, 1588-1592.
- 16) Reinherz, E.L., Cooper, M.D., Schlossman, S.F. & Rosen F.S. (1981) Abnormalities of T cell maturation and regulation in human beings with immunodeficiency disorders. *J. clin. Invest.*, **60**, 699-705.
- 17) Report of a WHO Scientific Group (1978) Immunodeficiency. *Technical Reports Series 630*, WHO, Geneva.
- 18) Sakai, O., Ralph, P., Cunningham-Rundles, C. & Good, R.A. (1982) Three distinct stages of B-cell defects in common variable immunodeficiency. *Proc. nat. Acad. Sci. USA*, **79**, 6008-6012.

- 19) Siegal, F.P., Siegal, M. & Good, R.A. (1978) Role of helper, suppressor and B-cell defects in the pathogenesis of the hypogammaglobulinemias. *New Engl. J. Med.*, **299**, 172-178.
 - 20) Stashenko, P., Nadler, L.M., Hardy, R. & Schlossman, S.F. (1980) Characterization of a human B lymphocyte-specific antigen. *J. Immunol.*, **125**, 1678-1685.
 - 21) Tsuchiya, S., Konno, T., Tada, K. & Ono, Y. (1980) Epstein-Barr virus-induced lymphoblastoid cell lines from patients with primary immunodeficiency diseases. *Scand. J. Immunol.*, **11**, 155-162.
 - 22) Tsuchiya, S., Nakae, S., Konno, T., Tada, K. & Ono, Y. (1981) Estimation of B-cells transformed by Epstein-Barr virus in patients with congenital agammaglobulinemia. *Tohoku J. exp. Med.*, **135**, 379-385.
 - 23) Tsuchiya, S., Minegishi, M., Imaizumi, M., Nakae, S., Tamura, M., Konno, T. & Tada, K. (1983a) Selective defect of OKT4⁺ T lymphocytes in severe immunodeficiency. *J. Pediat.*, **103**, 588-591.
 - 24) Tsuchiya, S., Sato, T., Nakae, S., Katamine, S., Konno, T. & Tada, K. (1983b) Epstein-Barr virus-induced precursor B cell lines from patients with congenital agammaglobulinemia. *Tohoku J. exp. Med.*, **140**, 133-144.
 - 25) Waldmann, T.A., Kurm, M., Broder, S., Blasckman, M., Blaese, R.M. & Strober, W. (1974) Role of suppressor T cells in pathogenesis of common variable hypogammaglobulinemia. *Lancet*, **2**, 609-613.
 - 26) Weiner, M.S., Bianco, C. & Nussenzweig, V. (1973) Enhanced binding of neuraminidase-treated sheep erythrocytes to human T lymphocytes. *Blood*, **42**, 939-946.
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