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Receptors for immunoglobulin isotype on T and B lymphocytes from untreated patients with chronic lymphocytic leukaemia

C. D. PLATSOUCAS, S. KEMPIN, A. KARANAS,* B. CLARKSON, R. A. GOOD & S. GUPTA *Memorial Sloan-Kettering Cancer Center, New York and* * *Department of Medicine, Roosevelt Hospital, College of Physicians and Surgeons, Columbia University, New York, USA*

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SUMMARY

Peripheral blood lymphocytes from eighteen untreated patients with chronic lymphocytic leukaemia (CLL) were analysed for the proportions of T and B lymphocytes with receptors for IgM, IgG or IgA. T lymphocytes with Fc receptors for IgM (Tμ cells) or IgA (Tα) cells were found in proportions comparable to those found in the controls. However, the proportion of T lymphocytes with receptors for IgG (Tγ cells) was significantly increased ($P < 0{\cdot}001$) resulting in an abnormally low ratio of Tμ/Tγ ($P < 0{\cdot}001$), when compared with normal controls. The proportion of B cells bearing Fc receptors for IgM, IgG or IgA was determined simultaneously. No significant differences were found between the normal controls and the patients with CLL. *In vitro* treatment of the purified T and B lymphocyte preparations with human leucocyte interferon, did not alter the proportions of the lymphocytes expressing Fc receptors for various immunoglobulin isotypes. The significance of these findings is discussed.

INTRODUCTION

Chronic lymphocytic leukaemia (CLL) is a lymphoproliferative disease usually of B cell origin (Preud'homme & Seligmann, 1972; Aisenberg & Block, 1972); however, a few cases of T cell CLL have been reported in the literature (Insel *et al.*, 1975; Yodoi, Takatsuki & Masuda, 1974). Patients with chronic lymphocytic leukaemia have normal T and B lymphocytes in the peripheral blood as well as leukaemic cells of B cell origin (Hersch *et al.*, 1971). The leukaemic B cells have monoclonal surface immunoglobulin, present in lower density than in normal B lymphocytes (Dickler *et al.*, 1973; Foulis, Cochran & Anderson, 1973). Furthermore, these cells form spontaneous rosettes with mouse red blood cells (Stathopoulos & Elliot, 1974; Gupta & Grieco, 1975), carry receptors for the C3d and C3b complement components (Ross *et al.*, 1973) and possess Fc receptors for immunoglobulin isotypes (Dickler *et al.*, 1973; Pichler & Knapp, 1978; Gupta, Platsoucas & Good, 1979b).

T lymphocytes in the peripheral blood of patients with CLL are present in low proportions, but in normal or elevated absolute numbers (Catovsky *et al.*, 1974). Purified T cells from these patients respond normally to mitogens (Han & Dadly, 1979). Peripheral blood lymphocytes from patients with CLL form significantly fewer T cell colonies *in vitro* in comparison with the normal controls (Dao *et al.*, 1978; Foa & Catovsky, 1979). In addition, T cells from certain patients with CLL were found to be defective in helper cell activity for the differentiation of B lymphocytes from normal donors to plasma cells (Fu *et al.*, 1978; Chiorazzi *et al.*, 1979).

Correspondence: Dr Chris D. Platsoucas, Memorial Sloan-Kettering Cancer Center, 1275 York Avenue, New York, New York 10021, USA.

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Recently human T lymphocytes have been divided into a number of subpopulations according to the presence of Fc receptors for immunoglobulin isotype. Tμ cells have receptors for IgM (Moretta *et al.*, 1975), Tγ cells for IgG (Dickler, Adkinson & Terry, 1974; Ferrarini *et al.*, 1975), Tα cells for IgA (Lum *et al.*, 1979*a*; Gupta *et al.*, 1979*c*) and *T*ε cells have receptors for IgE (Yodoi & Ishizaka, 1979; Gupta & Good, unpublished observations). The Tμ and Tγ cells are distinct with regard to morphology, and many effector and immunoregulatory functions, as reviewed by Gupta (1978), Gupta & Good (1978b), Moretta, Ferrarini & Cooper (1978). It has been shown that the Tμ cells contain a subpopulation of cells which act as helpers in the pokeweed mitogen-induced differentiation of B cells to immunoglobulin-synthesizing and -secreting plasma cells (Moretta *et al.*, 1977). In contrast, the Tγ cells contain a subpopulation of cells which act as suppressor in the same, PWM-induced system (Moretta *et al.*, 1977). Imbalance of the T cell subsets (Tμ and Tγ cells) have been reported in patients with primary and secondary immunodeficiencies (Gupta & Good, 1977b, 1978b; Gupta, Safai & Good, 1978b; Gupta & Tan, 1979; Trompeter, Layward & Hayward, 1978; Gupta *et al.*, 1979a). Although the function of the Tμ and Tγ cells has been elucidated in part, the functions of the newly described Tα cells are unknown.

Receptors for the Fc portion of IgM, IgG or IgA are not restricted to T lymphocytes and are present on B lymphocytes as well (Dickler & Kunkel, 1972; Ferrarini *et al.*, 1977; Pickler & Knapp, 1978; Gonzalez-Molina & Spiegelberg, 1977; Gupta *et al.*, 1979b; Lum *et al.*, 1979b).

In this paper we report the results of the analyses of the presence of receptors for IgM, IgG or IgA on T and B lymphocytes from untreated patients with CLL. A profound imbalance of the T cell subsets (Tμ and Tγ cells) was observed. Further more, we investigated the effect of *in vitro* treatment with human leucocyte interferon on the expression of Fc receptors on T and B lymphocytes for immunoglubulin isotype. Interferon has been shown in the past to augment the natural killer cell activity in humans (Einhörn, Blomgren & Strander, 1978; Trinchieri & Santoni, 1978; Herberman, Ortaldo & Bonnard, 1979), a property of at least some Tγ cells and of a population of non-T, non-B cells bearing Fc receptors for IgG (Kall & Koren, 1978; Gupta *et al.*, 1978a; Pichler, Gendelman & Nelson, 1979). Furthermore, interferon has been shown to increase selectively the expression on the cell surface, of histocompatibility antigens on normal lymphocytes and tumour cells (Vignaux & Gresser, 1977), thus resulting in alterations in the expression of cell surface phenotypes. However, here we were unable to demonstrate any alteration by *in vitro* treatment with human leucocyte interferon on the expression of Fc receptors for IgM, IgG or IgA on T and B lymphocytes, from either patients with CLL or normal donors.

MATERIALS AND METHODS

Patients. Eighteen untreated patients with CLL were the subjects of this study. Eighteen healthy, age- and sex-matched volunteers served as normal controls. Staging was determined by standard methods.

Preparation of T and B lymphocytes. Peripheral blood mononuclear leucocytes from patients with CLL and normal donors were isolated by centrifugation on a Ficoll–Hypaque (FH) cushion for 30 min at 400 ***g***, at room temperature. The cells were washed three times in Hanks' balanced salt solution (HBSS, Grand Island Biological, Grand Island, New York) and resuspended at a concentration of 4×10^6/ml in RPMI 1640 (Grand Island Biological) supplemented with 20% heat-inactivated foetal calf serum (Grand Island Biological). The mononuclear leucocytes were mixed at a ratio of 2:1 (v/v) with lymphocyte separator reagent (Technicon Instrument Co., Tarrytown, New York) and the mixture was incubated on a rotator for 30 min at 37°C. Phagocytizing monocytes were subsequently removed by centrifugation on a FH density cushion at room temperature for 20 min at 400 ***g***. Monocyte-depleted peripheral blood lymphocytes were removed from the interface, washed three times with HBSS and resuspended at a concentration of 4×10^6 cells/ml. T cells were prepared by rosetting with neuraminidase-treated sheep erythrocytes (Gupta *et al.*, 1978a; Platsoucas, Good & Gupta, 1979a). In brief, 2-ml aliquots of 1% neuraminidase-treated SRBC were mixed with 2 ml of lymphocyte suspension in HBSS and 0·5 ml of heat-inactivated and SRBC-absorbed foetal calf serum. The mixture was incubated at 37°C for 5 min, centrifuged at 200 ***g*** for 5

min and incubated for an additional hour at 4°C. The rosettes were carefully resuspended, incubated for an additional 15 min on ice, layered on FH and centrifuged for 20 min at constant temperature (22°C) at 400 *g*. Non-T cells were recovered from the interface and were found free of E rosette-forming cells. T lymphocytes were recovered from the pellets after lysis of the attached SRBC by Tris-buffered 0·83% ammonium chloride (pH 7·2) and were washed three times with HBSS. This population contained more than 95% T lymphocytes, as determined by rosetting with sheep erythrocytes and had virtually no cells with readily demonstrable surface immunoglobulin or non-specific esterase-positive cells. Non-T cells from patients with CLL were more than 95% B cells. From here on, these cells will be referred to as B cells. T and B cells were incubated overnight at 37°C in RPMI containing 20% foetal calf serum before determination of Fc receptors for Ig isotype.

Preparation of ox erythrocyte–antibody complexes. Rabbit IgG and IgM anti-ox erythrocyte antibodies were prepared as described previously (Gupta & Good, 1977a). IgM- and IgG-coated ox erythrocyte antibody complexes were prepared as follows (Gupta & Good, 1977a): washed 2% ox erythrocytes (Colorado Serum, Denver, Colorado) were mixed with equal volumes of purified rabbit IgM anti-ox erythrocyte antibody (1:20 dilution) or rabbit IgG anti-ox erythrocyte antibody (1:100 dilution) and incubated at room temperature for 2 hr. The complexes (EA_m and EA_g respectively) were washed three times with HBSS and resuspended at a concentration of 1%.

Ox RBC–TNP–IgA(ETα) complexes were prepared by the procedure described previously (Lum *et al.*, 1979a; Gupta *et al.*, 1979b; Platsoucas, Good & Gupta, 1980). One millilitre of packed ox erythrocytes was resuspended in a 10-ml buffer of 0·11 M Na_2HPO_4 (pH 7·4), supplemented with 100 mg of 2, 4, 6 trinitrobenzene sulphonic acid (TNP) and incubated with continuous mixing, for 30 min at room temperature. The cell suspension was washed once with cold phosphate-buffered saline (PBS), once with glycyl-glycine (0·6 mg/ml) and twice with phosphate-buffered saline and finally resuspended at a concentration of 2% in PBS. The trinitrophenyl-derived erythrocytes were coated with MOPC-315 IgA λ chain antibody (Litton Bionetics, Kensington, Maryland) which has binding capacity for TNP. A 2% TNP–RBC cell suspension was mixed with appropriate concentration (determined by titration) of IgA and incubated for 15 min at room temperature. The ET_a complexes were subsequently washed three times with HBSS and resuspended at concentration of 1%.

Determination of T and B lymphocytes with Fc receptors for IgM, IgG or IgA. One hundred microlitres of purified T or B lymphocytes (4×10^6 cells/ml) were mixed with an equal volume of 1% EA_m (for IgM receptor), EA_g (for IgG receptor) or ET_a (for IgA receptor), centrifuged at 200 *g* for 5 min and incubated at 4°C for 1 hr. The pellets were resuspended and 200 lymphocytes were counted. Lymphocytes with three or more erythrocytes attached were considered to be rosetting cells. T and B lymphocytes with receptors for IgA were counted under a fluorescent microscope, equipped with epi-illumination after the addition of a drop of 0·01% euchrycin. B lymphocytes from normal donors with receptors for IgG and IgA were determined by a double-labelling technique, for immunogloublin and Fc receptors as described previously (Gupta *et al.*, 1979b). The results were expressed as a percentage of total T or B lymphocytes.

Interferon treatment. Partially purified human leucocyte interferon was kindly provided by Dr Sohan L. Gupta. It was prepared by the method of Cantell *et al.*, (1974) and had a specific activity of approximately 7×10^5 units/mg of protein. Purified T or B cell preparations (3 to 5×10^6 cells/ml) were incubated with various concentrations of interferon for 14 hr at 37°C, in RPMI 1640 containing 20% foetal calf serum, 25 mM HEPES, 2 mM L-glutamine (Grand Island Biological) and penicillin–streptomycin. The cells were washed three times with HBSS and examined for their cell surface phenotypes.

RESULTS

The results of the analysis of the proportions of Tμ, Tγ and Tα cells from patients with CLL are shown in Table 1. Tμ/Tγ ratios and the staging of the disease are also shown in Table 1. The proportions of Tμ and Tα cells in patients with CLL were comparable to those of the normal controls. However, the proportions of the Tγ cells were found to be significantly increased

Table 1. Tμ, Tγ and Tα cells in untreated patients with CLL

Patients	Tμ (% cells)	Tγ (% cells)	Tα (% cells)	Tμ/Tγ	Stage
1*	39	38	4	1·03	IV
2	43	21	4	2·05	I
3*	46	75	3	0·59	IV
4	48	41	—	1·17	III
5	34	43	5	0·79	II
6	80	80	13	1·0	I
7	35	33	4	1·06	II
8	61	58	7	1·05	I
9	52	16	8	2·00	I
10	52	61	10	0·85	I
11	90	74	3	1·22	III
12	50	41	3	1·22	I
13	61	49	2	1·24	II
14*	18	47	9	0·38	III
15	48	12	19	4·00	I
16	40	43	7	0·93	I
17	27	8	8	3·37	I
18	44	19	3	2·31	I
Patients (mean ± s.e.m.)	48·25 ± 9·08	42·7 ± 5·0	7 ± 1	1·46 ± 0·22	
Normal controls (mean ± s.e.m.)	51·00 ± 2·03	13·05 ± 0·8	5 ± 1	4·18 ± 0·45	
Level of significance, patients *vs* controls	n.s.	<0·001†	n.s.	<0·001†	

* Treated.
† *P* value.

($P<0·001$) in patients with CLL when compared with those of the normal controls. Tμ/Tγ ratios were found to be significantly lower ($P<0·001$) in the patient group when compared with those of the normal controls. Furthermore, in six of eighteen patients the sum of the Tμ and Tγ cells was greater than 100% suggesting the existence of T cells bearing receptors for both IgM as well as IgG.

The proportions of B cells bearing Fc receptors for IgM, IgG or IgA were also determined. The results are shown in Table 2. No significant differences were found between the patients with CLL and the controls.

In vitro treatment of the T cells with various concentrations (0, 10, 100 and 1,000 iu/ml) of human leucocyte interferon did not alter the proportions of Tμ, Tγ or Tα cells either in the normal controls or the patients with CLL. Similarly, *in vitro* treatment of the B cells with human leucocyte interferon (0, 10, 100 and 1,000 iu/ml) did not alter the number of B cells expressing the Fc receptors for IgM, IgG or IgA, either in the CLL subjects or in the normal controls.

DISCUSSION

The present study demonstrates a profound imbalance of the Tμ and Tγ cells in untreated patients with B cell chronic lymphocytic leukaemia. Although the proportions of Tμ cells were comparable to those of normal donors, a significant ($P<0·001$) increase in the proportions of Tγ cells was observed in the patient group. No difference was found in the proportion of the Tα cells and the proportion of B cells bearing receptors for IgM, IgG or IgA. *In vitro* treatment of purified T and B

Table 2. Fc receptors for IgM, IgG and IgA on B cells from untreated patients with CLL

Patients	Fc (IgM)	Fc (IgG)	Fc (IgA)	Fc (IgM/IgG)	Stage
1*	62	51	6	1·99	IV
2	69	50	2	1·38	I
3*	46	88	2	0·52	IV
4	86	76	—	1·13	III
5	45	70	2	0·64	II
6	90	88	8	1·02	I
7	65	41	9	1·58	II
8	50	72	5	0·69	I
9	58	25	2	2·32	I
10	78	86	4	0·91	I
11	54	42	3	1·20	III
12	54	92	2	0·59	I
13	83	76	8	1·09	II
14*	59	81	4	0·73	III
15	12	15	11	0·80	I
16	90	86	10	1·05	I
17	66	61	7	1·08	I
18	59	71	13	0·83	I
Patients (mean ± s.e.m.)	62·56 ± 4·52	65·44 ± 5·39	5·76 ± 0·87	1·086 ± 0·113	
Normal controls (mean ± s.e.m.)	78·67 ± 7·88	74·00 ± 7·88	4·67 ± 0·67	1·063 ± 0·092	
Level of significance patients *vs* controls	n.s.	n.s.	n.s.	n.s.	

* Treated.

lymphocytes with human leucocyte interferon did not alter the expression of Fc receptors for immunoglobulin isotype.

The Tγ cells have been shown to include at least two subpopulations of T lymphoyctes. These cells contain a population of cells that are suppressors for the differentiation of B cells to plasma cells (Moretta *et al.*, 1977). Patients with CLL, although they contain high proportions of Tγ cells in the peripheral blood, do not appear to have abnormally high suppressor T cell activity (Fauci, Pratt & Whalen, 1977; Wolos & Davey, 1979). However, this finding has been challenged recently (Faguet, 1979). Furthermore, these patients frequently develop irreversible hypogammaglobulinaemia of variable severity and a profound antibody deficiency syndrome (reviewed by Gupta & Good, 1978a). Although it is believed that this hypogammaglubulinaemia may be attributed to an intrinsic defect of B cells to differentiate into immunoglublin-synthesizing and secreting plasma cells (Fauci *et al.*, 1977), the nature and origin of this secondary immunodeficiency is poorly understood. The role of the increased proportion of Tγ cells, observed in the present study, in the pathogenesis of the immunodeficiency in CLL remains to be elucidated. However, it is possible that increased proportions of Tγ cells, may reflect changes in distribution of this subpopulation from lymphoid tissue to the peripheral blood. It should be mentioned, however, that these Tγ cells do not appear to exhibit the suppressive and cytotoxic functions ascribed to this population. Furthermore, Fu *et al.* (1978) and Chiorazzi *et al.* (1979) have reported a helper T cell defect in the differentiation of normal B cells to plasma cells in the pokeweed mitogen-induced differentiation system in certain patients with CLL.

Tγ cells also contain a subpopulation of cells that are effectors in T cell-mediated spontaneous and antibody-dependent cytotoxicity (Gupta *et al*; 1978a; Kall & Koren, 1978; Pichler, Gendelman

& Nelson, 1979). In spite of the presence of increased proportions of Tγ cells, T cell-mediated spontaneous and antibody-dependent cytotoxicity have been found to be defective in patients with CLL (Platsoucas *et al.*, 1979b). *In vitro* treatment with human leucocyte interferon, although restoring the T cell-mediated cytotoxic activities in certain patients with CLL, failed to alter the expression of their cell surface phenotypes (unpublished results).

Our results also suggest that certain patients with CLL have significant proportions of T cells in their peripheral blood bearing Fc receptors for both IgM and IgG (Tμγ 'double' cells). The nature and function of these cells remains to be determined.

The proportions of B cells bearing Fc receptors for IgM, IgG or IgA were comparable to those of controls. An analysis of the Fc receptors for IgM, IgG and IgE of unfractionated leukaemic cells has been reported by Spiegelberg & Danier (1979). The function of these Fc receptors for immunoglobulins on B cells is not known, but feedback regulation of B cell responses by serum immunoglubulins at the isotype level has been proposed (Gupta *et al.*, 1979b). The B cells in patients with CLL contain two populations of B cells, the malignant and the normal B lymphocytes. The malignant B lymphocytes exhibit faint staining by immunofluorescence and possess decreased amounts of cell surface monoclonal Ig, usually of the IgM isotype, with or without IgD (Koziner *et al.*, 1977). It is possible that leukaemic B cells could suppress antibody responses of normal B cells in patients with CLL. This suppression, if it occurs, could be responsible for the hypogammaglobulinaemia so commonly observed in these patients. Recently we were able to separate at least two subpopulations of B cells in CLL by density gradient electrophoresis on the basis of their surface charge (unpublished results in collaboration with Dr Andreeff). Functional analysis of these subpopulations is in progress.

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