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ANTIBODY-DEPENDENT LYMPHOCYTE KILLER FUNCTION IN HUMAN IMMUNODEFICIENCY DISEASES

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SUMMARY

Antibody-dependent cell immunity to the lymphocyte system (ABCIL) has been shown to be a function of a non-thymus-processed cell in the experimental animal. To evaluate its role in the human and to assess its clinical usefulness, we assessed ABCIL in twenty-five patients with various immunodeficiency (ID) syndromes.

Our technique measures the lysis of ^{51}Cr-labelled normal human lymphocytes coated with HL-A-specific antibody. Cytotoxicity is expressed as a percentage of ^{51}Cr released after subtracting the spontaneous target cell release. Mean values in normals are 20 ± 2 (s.e.).

The ten patients with AB deficiency had a mean ABCIL of $7{\cdot}9 \pm 2$ ($P < 0{\cdot}01$). All eight patients with cellular ID had normal ABCIL (18 ± 2), while the ten patients with combined ID had variable results.

Effector cell function in the ABCIL test correlated ($r = 0{\cdot}74$; $P < 0{\cdot}05$) with the percentage of B cells in the peripheral blood. No correlation was found between ABCIL function and serum immunoglobulin levels or rosette-forming cells in the peripheral blood. There is a function for B lymphocytes other than as a precursor of antibody-synthesizing cells.

INTRODUCTION

The *in vitro* destruction of antibody-coated target cells by isolated, non-sensitized lymphocytes often termed antibody-dependent cell-mediated cytotoxicity or antibody-dependent cell immunity to the lymphocyte system (ABCIL) (McConnachie & Dossetor, 1973) was first described by Möller (1965) utilizing sarcoma cells as a target for mouse lymphocytes. Subsequent studies have indicated that the antibody involved is an IgG globulin (Möller & Svehag, 1972) and that complement is not involved in cell destruction (Perlmann & Perlmann, 1970); further, various target cells can be used including tumour cells, cultured fibroblasts and xenogeneic erythrocytes (Perlmann & Holm, 1969).

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Prior studies in animal systems implicate the B (bursa-derived) lymphocyte as the effector cell in ABCIL (Harding *et al.*, 1971; MacLennan & Harding, 1970; Van Boxel *et al.*, 1972).

Studies of this system in human (Möller & Svehag, 1972; Blaese, Rosenberg & Wunderlich, 1972; Wisloff & Froland, 1973) have shown divergent results as to the type of cell mediating this reaction. In order to extend this area of study and to determine the clinical applicability of this procedure, we performed ABCIL in twenty-five patients with well-defined primary immunodeficiency syndromes with defects of cellular or humoral immunity or both. We compared the results of this procedure with other antibody and cellular immune tests used to define primary immunodeficiencies.

PATIENTS

Twenty-five patients with various immunodeficiency diseases (Table 1) were studied, including ten patients with antibody deficiency (cases 1–10), eight with cellular deficiency (cases 11–18) and seven with combined deficiencies (cases 19–25).

There were two patients with X-linked agammaglobulinaemia (Bruton), three with adult-onset hypogammaglobulinaemia, three with severe combined immunodeficiency (all with cellular immunity reconstitution; one by bone marrow and two by foetal thymus transplantation), two with antibody deficiency with hyper-IgM, three patients with cellular immunodeficiency with immunoglobulins (Nezelof's syndrome), four with chronic mucocutaneous candidiasis, one with thymic hypoplasia (DiGeorge syndrome), five with ataxia telangiectasia, two with immunodeficiency with thrombocytopenia and eczema (Wiskott–Aldrich syndrome (WAS)). These were compared with thirty-four normal subjects; fourteen less than 1 year, eight between 1 and 6 years and eighteen of 7–50 years of age.

MATERIALS AND METHODS

IgG, IgM and IgA determinations were performed by single radial immunodiffusion (Stiehm & Fudenberg, 1966). B cells were determined by detecting membrane-bound immunoglobulin by indirect immunofluorescence using anti-immunoglobulin antiserum (Greaves, 1970). Normal values are between 14 and 20%.

Delayed hypersensitivity skin tests performed on most patients included 5 units of purified protein derivative (Connaught, Englewood, New Jersey), 1:10 monilia (Hollister-Stier, Burbank, California), 250–1000 units of streptokinase/25–250 units of streptodornase (Lederle, Pearl River, New York), mumps skin test antigen (0·1 ml) (Eli Lilly, Indianapolis, Indiana), and 1 or 10 μg of phytohaemagglutinin (Lawlor *et al.*, 1973).

Heparinized venous blood was obtained from each patient and lymphocytes were isolated by Ficoll–Hypaque flotation (Böyum, 1968). *In vitro* reactivity of lymphocytes to phytohaemagglutinin (Difco Laboratories, Detroit, Michigan) was performed by addition of PHA-M to $0{\cdot}1 \times 10^6$ of isolated peripheral lymphocytes and cultured by the microtechnique of Sengar & Terasaki (1971).

T cells were detected by the ability of the lymphocytes to form rosettes with sheep erythrocytes (Wybran *et al.*, 1973). Normal values for rosette-forming cells (RFC) is 60–80%.

Lymphocytes were typed for HL-A antigens (Mittal *et al.*, 1968) and tested, as previously described (McConnachie & Dosseter, 1973), against one or more ^{51}Cr-labelled resting normal lymphocyte target cells coated with an allogeneic HL-A antibody specific to the target.

Antibodies were previously tested for the ability to cause lymphocyte-mediated cytolysis of targets.

Specific details of the micro ^{51}Cr release test are as follows. Target cells were labelled with ^{51}Cr (50–100 μCi) (Brunner *et al.*, 1968), washed five times in McCoy's 5a medium and suspended at a concentration of $1{\cdot}5 \times 10^5$ cells/ml. Aliquots of 0·05 ml of target cells and antibody (diluted 1:5 in McCoy's 5a) were mixed in polystyrene tubes (Fisher, 1·4 ml). Effector cells (0·2 ml of 6×10^5 cells/ml suspension in McCoy's 5a) were added with mixing. The total mixture was aliquotted into three polyethylene tubes (Beckman, 0·4 ml), centrifuged briefly to promote contact, and incubated at 37°C for 4 hr.

All antibodies were treated at 56°C for 30 min and clarified by centrifugation before use. To determine ^{51}Cr release due to target damage, 0·1 ml of medium was added to each tube, mixed, and centrifuged for 2 min. The supernatant medium containing released ^{51}Cr was transferred to a second tube and both tubes were counted in a gamma spectrometer (Nuclear Chicago). Released ^{51}Cr was divided by the total of released and cell-contained ^{51}Cr to obtain a percentage ^{51}Cr release of the total for each of the three aliquots. These values were averaged, the standard error of each test was calculated, and the mean test release was adjusted for the mean spontaneous ^{51}Cr release of the target cell by subtraction.

Spontaneous release was that obtained by incubating the target cell in the presence of autologous effector cells in medium with autologous plasma in place of antibody. This was also done in triplicate; means and standard errors were calculated.

Thus, the results recorded are the differences between test and spontaneous ^{51}Cr release for each effector lymphocyte and one or more antibody-coated targets.

Most patients were tested on three to five different days with minimal variability of test results. Two patients with ataxia telangiectasia (case 19 and case 20) were tested on one occasion.

RESULTS

Deficient effector cell function in the ABCIL test was strikingly correlated with deficiency of circulating B cells. Patients with antibody immunodeficiency and combined antibody–cellular immunodeficiencies demonstrated decreased effector cell function, but patients with isolated cellular defects demonstrated effector cell function comparable to control values (Fig. 1).

Seven of the ten antibody immunodeficiency patients had deficient ABCIL ability. These included five patients with agammaglobulinaemia (cases 1–5) (Table 1) whose ABCIL values ranged from 0·3 to 9%; mean $3{\cdot}7 \pm 1{\cdot}4$ ($P < 0{\cdot}01$). All of these patients had reduced amount of B cells.

Case 6 has SCID which was partially reconstituted with a bone marrow transplant from an HL-A identical sibling. His delayed type hypersensitivity skin tests and *in vitro* lymphocyte stimulation by PHA are normal but he remains hypogammaglobulinaemic with 5% B cells and deficient ABCIL. Cases 7 and 8 have SCID; both had reconstitution of cellular immune function after engraftment of intraperitoneally administered foetal thymus. Both remain hypogammaglobulinaemic; case 7 has 1% B cells with deficient ABCIL (5 ± 1) while case 8 has 10% B cells and normal ABCIL (17 ± 1).

The two patients (cases 9 and 10) with immunodeficiency with hyper-IgM had normal number of B cells (16 and 20%), normal cellular immune function and normal ABCIL (19 ± 3 and 16 ± 3).

TABLE 1. Antibody and cellular immune studies in twenty-five immunodeficiency patients

Case	Diagnosis	Age (years)	Sex	Immunoglobulins (mg%)			B cell (%)	T cell* (%)	*In vitro* PHA*	Skin* test	Antibody-mediated cell-dependent immune lympholysis (%^{51}Cr)	
				IgG	IgM	IgA					$\bar{X} \pm$ s.e.	
	Antibody immunodeficiency											
1	X-linked agammaglobulinaemia	5	M	248	41	14	4	75	N	N	9	1
2	X-linked agammaglobulinaemia	18	M	220	68	28	1	82	N	N	4	2
3	Adult hypogammaglobulinaemia	42	F	310	50	20	5	68	N	N	0·3	0·2
4	Adult hypogammaglobulinaemia	29	F	200	70	62	8	80	N	N	0·6	0·6
5	Adult hypogammaglobulinaemia	50	F	220	70	40	3	63	N	N	5	2
6	Bone marrow Rx SCID†	4	M	392	80	37	5	n.d.	N	N	3	2
7	Thymus Rx SCID†	6/12	M	252	35	0	1	45	N	N	5	1
8	Thymus Rx SCID†	1·5	F	220	80	0	10	52	N	N	17	1
9	Antibody deficiency with hyper-IgM	16	M	682	300	11	16	43	N	N	19	3
10	Antibody deficiency with hyper-IgM	4	F	585	230	23	20	78	N	N	16	3
	Total										7·9	2·1
	Cellular immunodeficiency											
11	Nezelof's syndrome	16	M	720	110	82	22	n.d.	A	A	24	5
12	Nezelof's syndrome	3	F	800	100	60	n.d.	n.d.	A	A	14	2
13	Nezelof's syndrome	6	M	890	130	120	18	n.d.	A	A	24	3
14	Chronic mucocutaneous candidiasis	8	M	1600	90	64	26	37	N	N	17	1

15	Chronic mucocutaneous candidiasis	1	F	1509	150	210	22	75	N	N	23	1
16	Chronic mucocutaneous candidiasis	10	F	1942	145	56	n.d.	n.d.	N	N	14	2
17	Chronic mucocutaneous candidiasis	14	F	1624	122	146	19	39	N	A	19	2
18	DiGeorge syndrome	3/12	M	320	39	4·5	16	4	A	A	10	2
	Total										18·1	1·7
	Combined immunodeficiencies											
19	Ataxia telangiectasia	8	M	1600	183	3	29	70	N	N	33	—
20	Ataxia telangiectasia	7	M	900	72	0	n.d.	30	A	A	49	—
21	Ataxia telangiectasia	14	F	1261	214	145	20	37	N	N	22	2
22	Ataxia telangiectasia	16	M	886	125	78	27	48	N	N	18	2
23	Ataxia telangiectasia	9	M	1385	260	112	20	48	n.d.	N	18	2
24	Wiskott–Aldrich syndrome	4	M	1807	48	143	20	n.d.	A	A	0·6	0·4
25	Wiskott–Aldrich syndrome	7	M	735	43	130	16	40	A	A	0·3	0·3
	Total										20·6	5·3
	Normal values:			Age-related‡			14–20	60–80	0–6 months		10·2	1·2
									7–12 years		9·2	1·6
									1–6 years		21·8	2·3
									7–50 years		20·5	1·7
									Age-related§			

n.d. = Not done.

* N = normal; A = abnormal.

† SCID = severe combined immunodeficiency.

‡ Stiehm & Fudenberg (1966).

§ McConnachie *et al.* (1973).

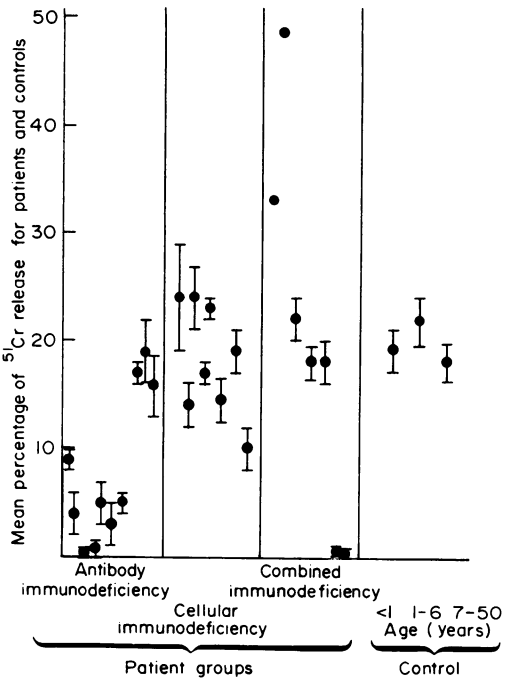

FIG. 1. Effector cell function in the ABCIL system; immunodeficient patients and controls. Each point represents the mean percentage of ^{51}Cr release of one individual tested against one or more allogeneic lymphocyte targets sensitized with anti-HL-A antibody specific to the target. Patient results are shown in order of case number from the left. Control group divided by age: < 1 year (eight points); 1–6 years (eight points); 7–50 years (eighteen points). Bars represent one s.e. of mean for each age group.

As a group the ten patients with serum antibody immunodeficiency had a mean ABCIL of $7{\cdot}9 \pm 2{\cdot}1$ ($P < 0{\cdot}01$).

All eight patients with isolated cellular defects (cases 11–18) had normal ABCIL with a range of 10–24% and a mean of $18{\cdot}1 \pm 1{\cdot}7$ (s.e.). The lowest value was noted in a 3-month-old with thymic hypoplasia (case 18) but a value of 10% is normal killer function for this age (McConnachie *et al.*, 1973) in this system. All of these patients had normal numbers of B cells and/or intact antibody function. The three patients with cellular immunodeficiency with immunoglobulins (Nezelof's syndrome) and the patient with thymic hypoplasia (DiGeorge's syndrome) had negative delayed type hypersensitivity skin tests and abnormal *in vitro* responses to PHA. Of the four patients with chronic mucocutaneous candidiasis, two had decreased percentage of RFC; their lymphocytes responded normally to *in vitro* PHA stimulation.

Among the seven (cases 19–25) patients with combined antibody and cellular immunodeficiency variable ABCIL effector cell function was observed with a range of 0·3–49%. The five patients with ataxia telangiectasia (cases 19–23) had normal ABCIL (mean of $28 \pm 5{\cdot}9$); four of these had a normal percentage of B cells (one wasn't done); two were IgA-deficient, though the other immunoglobulins were normal; four had decreased numbers of

RFC with one having abnormal skin tests and *in vitro* PHA response (case 20). Patients with Wiskott–Aldrich syndrome (cases 24–25) had depressed ABCIL (0·3 and 0·6%); both had a normal percentage of B cells, normal Ig levels, but non-functioning IgM. Skin tests and *in vitro* PHA stimulation was abnormal and one had decreased peripheral T cells.

There appears to be a correlation ($r=0·74$; $P<0·05$) between effector cell function in the ABCIL test and the percentage of B cells in the peripheral blood as determined by indirect immunofluorescence of membrane-bound immunoglobulins (Fig. 2). Only the two patients with WAS had a normal percentage of B cells with deficient ABCIL function. No such correlation was found between effector cell function in the ABCIL test and serum immunoglobulin levels or rosette-forming (thymus-dependent) cells in peripheral blood.

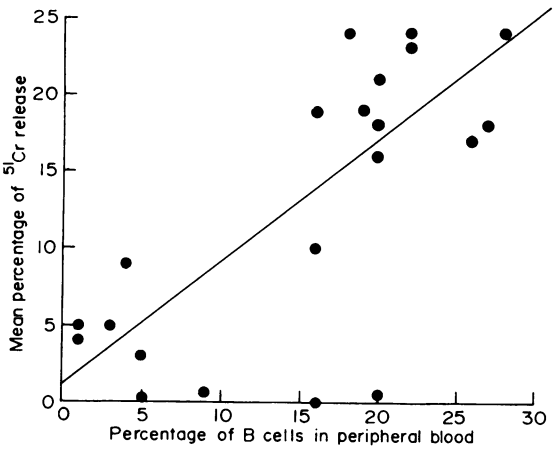

FIG. 2. Correlation of effector cell function in ABCIL and percentage of B cells in peripheral blood in twenty immunodeficient patients. ($r=0·74$; $P<0·05$.)

DISCUSSION

Patients with B-cell defects are the second group of human subjects with deficient ABCIL. Our previous study (McConnachie *et al.*, 1973) documented deficient ABCIL in very young individuals and to a lesser extent in aged individuals. In both the immunodeficiency subjects and newborns T-cell function is intact, suggesting that ABCIL is a B-cell function.

Blaese *et al.* (1972) studied the characteristics of human lymphocytes participating in cytotoxic reactions promoted by one lymphocyte-dependent antibody (LDA), using allogeneic lymphocytes as targets. Patients with intestinal lymphangiectasia had normal function in their system. These individuals lose recirculating long-lived lymphocytes (T cells) and have markedly depressed cellular immune function with normal antibody function. The lymphocytes from the chylous pleural effusions of these patients had depressed antibody mediated lymphocytotoxicity and normal response to *in vitro* PHA stimulation. These cells, which enter the effusion directly with chylous lymph, would be predominantly recirculating (T cell) lymphocytes. The nine patients with hypogammaglobulinaemia studied by these authors had reduced (50% of normal) ability to mediate killer function. In contrast to our results, the mean cytotoxicity (95%) for seven patients with WAS was normal with a range of 35–155% of normal.

Wisloff & Froland (1973), using a xenogeneic target system (^{51}Cr-labelled chicken red blood cells and rabbit antichicken red blood cell antibodies) reported that antibody-induced cell-mediated cytotoxicity in man is independent of B lymphocytes. Normal lymphocytes after filtration on nylon-wool columns (i.e. 'pure' T lymphocytes) manifested intact cytotoxic function. Three patients with X-linked hypogammaglobulinaemia gave similar results. However, there is some disagreement whether passage through nylon-wool columns deplete human immunoglobulin-bearing lymphocytes (Dickler & Kunkel, 1972).

It would be difficult to compare our results with the above since their test system involves a xenogeneic model while ours uses only human allogeneic lymphocytes. Newborns have been shown to lyse antibody-coated chicken red blood cells (Stites *et al.*, 1971), while they are deficient in our system.

Our data, showing a direct correlation of the B-cell population with the ability of an individual's lymphocytes to manifest intact ABCIL, suggests a primary role for the B lymphocyte. Though the mechanism remains unclear, one can postulate from other investigations (Basten *et al.*, 1972; MacLennan, 1972) that initial interaction between effector cell and antibody-coated target occurs at the Fc receptor on B lymphocytes.

The 'super' killer function of some of the ataxia-telangiectasia patients is presently unexplainable. One could suppose that there exists a large or more efficient population of effector cells.

That this effector function is abnormal in newborns, patients with hypogammaglobulinaemia and the Wiskott–Aldrich syndrome and intact in patients with antibody immunodeficiency with hyper-IgM suggests that normal production of IgM is required. However, since IgM antibody is ineffective in mediating this reaction (Carlsson *et al.*, 1971), a role for cell surface IgM is suggested.

Our data does not prove association of ABCIL effector function with B cells but it does associate deficient function with decreased B-cell content of peripheral blood. It is possible that the effector cell in ABCIL is neither a B cell nor a T cell but a non-immunoglobulin carrying 'null' cell which is depleted along with B cells, or is rendered less capable of functioning by depletion of immunoglobulin-bearing cells.

In any event, there appears to exist a function(s) for B lymphocytes other than as a precursor of antibody-synthesizing cells. As yet, there is no *in vivo* function known for ABCIL effector cells; potential candidates could be graft rejection, autoimmune disease and possibly immune surveillance.

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REFERENCES

BASTEN, A., MILLER, J.F.A.P., SPRENT, J. & PYE, J. (1972) A receptor for antibody on B lymphocyte. I. Method for detection and functional significance. *J. exp. Med.* **135**, 610.

BLAESE, R.M., ROSENBERG, E. & WUNDERLICH, J. (1972) Evidence of two functionally distinct lymphocyte populations in man. *Microenvironmental Aspects of Immunity* (ed. by B. D. Jankovic & K. Isakovic), p. 315. Plenum, New York.

BÖYUM, A. (1968) Separation of lymphocytes from blood and bone marrow. *Scand. J. clin. Lab. Invest.* **21,** supplement 97.

BRUNNER, K.T., MAVEL, J., CEROTTINI, J.C. & CHAPUIS, B. (1968) Quantitative assay of the lytic action of immune lymphoid cells on ^{51}Cr-labelled allogeneic target cells *in vitro*. *Immunology*, **14,** 181.

CARLSSON, H.E., HAMMARSTRÖM, S., LANTELI, M. & PERLMANN, P. (1971) Antibody-induced destruction of colon antigen-coated chicken erythrocytes by normal human lymphocytes. *Europ. J. Immunol.* **1,** 281.

DICKLER, H.B. & KUNKEL, H.G. (1972) Interaction of aggregated γ-globulin with B lymphocytes. *J. exp. Med.* **136,** 191.

GREAVES, M.F. (1970) Biological effects of anti-immunoglobulins: evidence for immunoglobulin receptors on 'T' and 'B' lymphocytes. *Transplant. Rev.* **5,** 45.

HARDING, B., PUDIFIN, D.J., GOTCH, F. & MACLENNAN, I.C.M. (1971) Cytotoxic lymphocytes from rats depleted of thymus processed cells. *Nature: New Biology*, **232,** 80.

LAWLOR, G.J., STIEHM, E.R., KAPLAN, M.S., SENGAR, D.P.S. & TERASAKI, P.I. (1973) Phytohemagglutinin (PHA) skin test in the diagnosis of cellular immunodeficiency. *J. Allergy*, **52,** 31.

MACLENNAN, I.C.M. (1972) Competition for receptors for immunoglobulin on cytotoxicity lymphocytes. *Clin. exp. Immunol.* **10,** 275.

MACLENNAN, I.C.M. & HARDING, B. (1970) Failure of certain cytotoxic lymphocytes to respond mitotically to phytohemagglutinin. *Nature* (*Lond.*), **227,** 1246.

MCCONNACHIE, P.R. & DOSSETER, J.B. (1973) Antibody-mediated cell-dependent immunity in human lymphocytes. *Tissue Antigens*, **3,** 303.

MCCONNACHIE, P.R., RACHELEFSKY, G.S., STIEHM, E.R. & TERASAKI, P.I. (1973) Antibody-dependent lymphocyte killer function and age. *Pediatrics*, **52,** 795.

MITTAL, K.K., MICKER, M.R., SINGAL, D.P. & TERASAKI, P.I. (1968) Serotyping for homotransplantion. XVIII. Refinement of micro-droplet lymphocyte cytotoxicity test. *Transplantation*, **6,** 913.

MÖLLER, E. (1965) Contact induced cytotoxicity by lymphoid cells containing foreign isoantigens. *Science*, **147,** 873.

MÖLLER, G. & SVEHAG (1972) Specificity of lymphocyte-mediated cytotoxicity by *in vitro* antibody-coated target cells. *Cell. Immunol.* **4,** 1.

PERLMANN, P. & HOLM, G. (1969) Cytotoxic effects of lymphoid cells *in vitro*. *Advanc. Immunol.* **11,** 117.

PERLMANN, P. & PERLMANN, H. (1970) Contactual lysis of antibody-coated chicken erythrocytes by purified lymphocytes. *Cell. Immunol.* **1,** 300.

SENGAR, D.P.S. & TERASAKI, P.I. (1971) A semimicro mixed leukocyte culture test. *Transplantation*, **11,** 260.

STIEHM, E.R. & FUDENBERG, H.H. (1966) Serum levels of immunoglobulins in health and disease: a survey. *Pediatrics*, **37,** 715.

STITES, D.P., WYBRAN, J., CARR, M.C. & FUDENBERG, H.H. (1971) Development of cellular immunocompetence in man. *Ontogeny of Acquired Immunity*. CIBA Foundation Symposium (23–25 November 1971), p. 113. ASP, Amsterdam.

VAN BOXEL, J.A., STOBO, J.D., PAUL, W.E. & GREEN, I. (1972) Antibody-dependent lymphoid cell-mediated cytotoxicity: no requirement for thymus-derived lymphocytes. *Science*, **175,** 194.

WISLOFF, F. & FROLAND, S.S. (1973) Antibody-dependent lymphocyte-mediated cytotoxicity in man: no requirement for lymphocytes with membrane-bound immunoglobulin. *Scand. J. Immunol.* **2,** 151.

WYBRAN, J., LEVIN, A.S., SPITLER, L.E. & FUDENBERG, H.H. (1973) Rosette forming cells, immunologic deficiency diseases and transfer factor. *New Engl. J. Med.* **288,** 710.