Antibody-Dependent Lymphocytotoxicity Induced by Immunoglobulin G from Hodgkin's Disease Splenic Lymphocytes

Abstract. *Immunoglobulin G, produced in cultures of splenic lymphocytes obtained from patients with Hodgkin's disease, bound to a population of homologous peripheral blood lymphocytes and initiated antibody-dependent cell cytotoxicity in cultures from five out of eight patients. Two patients whose cultures produced negative results had minimal disease; the other was in remission. The target cells appear to be T lymphocytes; the effector cells bear Fc receptors that are inhibited by antigen-antibody complexes. Antibody-dependent cell cytotoxicity events may produce the anergy and lymphopenia often seen in Hodgkin's disease.*

Depression of thymus-derived lymphocyte (TL) function in Hodgkin's disease has been extensively documented (*1*). Bone marrow–derived lymphocyte (BL) function remains intact until the disease is far advanced (*2*), although primary antigenic stimulation responses, which require TL cooperation, may be deficient (*3*). It has been proposed that the disease, which is often manifested by early anergy, is associated with a TL malignancy (*4*), or that serum factors are operative which suppress TL function and cell-mediated immune responses (*5*). Others postulate that an interaction between normal and antigenically altered TL's (*6*), similar to a chronic graft versus host disease (*7*) eventuates in loss of normal TL function.

Previously, we demonstrated an increased production of immunoglobulin G (IgG) by cultured splenic lymphocytes from patients with Hodgkin's disease; a quantitatively detectable increase of IgG at cell surfaces occurred after exposure of homologous peripheral blood lymphocytes (PBL's) to the secreted IgG (*8*). An antibody response to antigenically altered TL's with cross-specificity to "nonmalignant" TL's has been suggested (*9*) as causing the follicular hyperplasia, hyperreactivity of BL's (*8*), and relative unresponsiveness of circulating lymphocytes to phytohemagglutinin (*10*). We now present autoradiographic evidence that IgG produced in cultures of splenic lymphocytes obtained from patients with Hodgkin's disease specifically binds to, and initiates antibody-dependent cell cytotoxicity (ADCC) of, normal homologous PBL's. These findings may explain the depression of TL function in vivo in Hodgkin's disease and may be of significance in the pathogenesis of the disease.

Immunoglobulin G was isolated as described (*8*) from cultures of splenic lymphocytes obtained from normal subjects, patients with Hodgkin's disease, and patients with non-Hodgkin's lymphomas (non-HL). Splenic tissues, obtained at surgery, were minced in Kontes grinders and suspended in RPMI 1640. Filtered cell suspensions were washed four times and cultured in RPMI 1640 containing 20 percent fetal calf serum, at a concentration of 2×10^6 mononuclear cells per milliliter, for 10 days at 37°C. At day 5, one-half of the culture supernatant was aspirated and replaced with fresh medium. At day 10, the cultures and media were pooled and taken to 50 percent saturation with ammonium sulfate. They were then centrifuged, and the precipitate was resuspended, dialyzed, and eluted from DEAE-Sephadex with 0.01*M* sodium phosphate buffer, *p*H 8.0. The eluate was concentrated, tested for purity by immunoelectrophoresis, and quantitatively assayed by the ^{125}I-labeled Fab antigen-antibody method (*8*). The antibody to Fab was standardized so that 0.5 ml precipitated 50 percent of the radioactivity in 0.5 ml of an antigen solution containing ^{125}I-labeled Fab (20.0 ng of nitrogen). The ability of the test materials to inhibit this reaction was compared to known amounts of pooled, normal human IgG.

The IgG produced in this manner by cells from patients with Hodgkin's disease binds to normal PBL's, whereas IgG produced by cells from normal subjects and non-HL patients does not. The IgG was labeled radioactively with ^{125}I by the lactoperoxidase method (*11*). The binding of [^{125}I]IgG lacked uniformity from sample to sample. In some instances, a subpopulation of lymphocytes appeared to act as targets (Fig. 1a), while intense labeling of a larger percentage of lymphocytes occurred with other samples of IgG (Fig. 1b). Cell death of the targeted lymphocytes could be seen in some instances as early as 1 hour after exposure to IgG from cells of patients with Hodgkin's disease; and cell death appeared to reach a maximum at varying intervals of time up to 18 hours. All five samples of [^{125}I]IgG derived from the patients with Hodgkin's disease gave autoradiographic evidence of binding to homologous PBL's. In contrast, [^{125}I]IgG from the spleens of four non-HL patients and from four normal subjects failed to show this phenomenon. When enriched populations of BL's, prepared as described (*12*), were used as targets for Hodgkin's derived [^{125}I]IgG, these also failed to show demonstrable autoradiographic binding, suggesting that a TL-associated antigen is necessary for the IgG interaction.

To measure the effect of the IgG from patients with Hodgkin's disease on lymphocytes, an ADCC test was used (*13*) (Table 1). Peripheral blood lymphocytes were isolated as described (*12*), and homologous PBL's were used as both

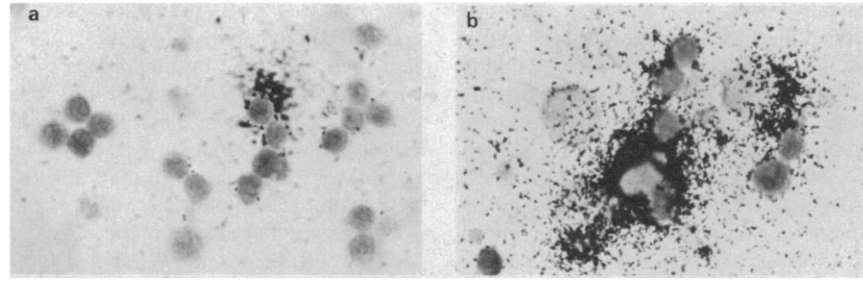

Fig. 1. Autoradiographs of homologous PBL's. (a) One hour and (b) 18 hours after exposure to [^{125}I]IgG (400 ng of nitrogen) derived from cultured splenic lymphocytes from patients with Hodgkin's disease. The lymphocytes were obtained by centrifugation (1050*g*, 20 minutes) of peripheral blood layered over Hypaque-Ficoll. The cells were collected at the interface and washed four times with RPMI 1640 prior to incubation with the IgG samples. Iodine-25–labeled IgG (400 ng of nitrogen) was added to 3×10^6 homologous PBL in 1 ml of RPMI 1640. After varying periods of incubation at 28°C, samples of cells were placed on gelatin-coated slides and fixed with methanol. Kodak nuclear track emulsion (NTB-2) was added and the slides were stored for 1 to 3 days prior to development and staining with a standard Wright's solution. In certain instances, enriched B lymphocyte populations (*12*) were used as target cells. Sheep red blood cells (RBC's) (2 percent), previously treated with 2-aminoethylisothiouronium bromide hydrobromide (AET), were added at equal volume to 1×10^7 PBL per milliliter. After centrifugation at 200*g* for 8 minutes and incubation at 4°C for 1 hour, the cell pellet was gently resuspended in RPMI 1640, layered over Hypaque-Ficoll, and centrifuged for 20 minutes at 1050*g*. Of the cells that did not form rosettes but collected at the interface, 92 percent showed membrane bound immunoglobulin and 2 percent formed rosettes when AET-treated sheep RBC's were added. A small proportion of lymphocytes in (a) showed intense labeling; targeted cells in (b) appeared dead and clumped (×670).

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Table 1. Antibody-dependent cell cytotoxicity test of homologous PBL's by IgG produced in splenic lymphocyte cultures. The cytotoxic index is the difference between the percentage of ^{51}Cr released in experimental and control tubes divided by 100 minus the percentage of ^{51}Cr spontaneously released in control tubes. Abbreviations: wd, well differentiated; pd, poorly differentiated.

Source of IgG	Spleen involvement	Normal donor (target and effector source)	IgG per 10^6 lymphocytes (nanograms of nitrogen)	Cytotoxic index
		Control subjects		
Normal		R.L.	200	0.0
Normal		S.R.	200	0.0
Spherocytosis		W.P.	1000	0.0
Spherocytosis		B.W.	200	0.0
Ruptured spleen		D.S.	432	1.4
		Patients with non-Hodgkin's lymphomas		
Diffuse, wd lymphocytic		R.L.	180	0.0
Diffuse, pd lymphocytic		W.P.	400	0.0
Nodular, pd lymphocytic		S.R.	200	0.0
Diffuse, histiocytic		R.G.	250	0.7
		Patients with Hodgkin's disease		
H3, at stage IA	No	D.S.	130	51.0
H5, at stage IA		G.L.	130	39.2
H5, at stage IA	No	R.L.	270	44.5
H5, at stage IA		S.R.	500	29.3
H43, at stage IIIB	Yes	R.L.	70	22.0
H65, at stage IA*	No	S.R.	170	3.0
H132, at stage IIIA	Yes	P.G.	130	10.0
H132, at stage IIIA		W.P.	330	20.0
H7, at stage IVB	No	R.L.	300	0.0
H22, at stage IA	No	G.L.	250	0.0
H75, at stage IA	No	B.W.	200	0.0

*Unilateral cervical involvement.

target and effector cells. After they were washed three times with RPMI 1640, 1×10^8 lymphocytes were labeled by the addition of 100 μc of ^{51}Cr for 1 hour at 37°C. The PBL's were then washed three times, and suspended in Fisher tubes, each containing 3×10^6 lymphocytes; control and experimental IgG of known quantity was then added. After incubation at 25°C for 18 hours, the tubes were centrifuged at 3000*g* for 1 minute and the radioactivity in the supernatants was measured in an automated gamma counter. The IgG from five out of eight patients with Hodgkin's disease induced ADCC of homologous PBL. Cells from the five patients with positive autoradiographic results also gave positive results in the ADCC test. In those patients with negative results in the ADCC test, the spleen was not affected by the disease. In two of these patients the disease was at stage IA and there was involvement of a single cervical focus. In the third patient the disease was at stage IVB; this patient had completed a 6-month course of chemotherapy with nitrogen mustard, vincristine, procarbazine and prednisone less than 1 month before splenectomy, and at the time of the operation, no evidence of Hodgkin's disease could be found.

The binding of antibody to a target cell may initiate lysis of the cell by one of two mechanisms. Either the fixing of complement by antibody Fc (crystallizable fragment of IgG) pieces causes activation of the complement cascade and subsequent lysis of the cell, or effector cells with Fc receptors combine with antibody at the target cell surface to induce lysis (*13*). The nature of the effector cell, in the complement-free ADCC system, was examined by inhibiting Fc receptors of potentially cytotoxic cells complexes of human Fab antigen and rabbit antibody to human Fab (*14*). The complexes, at slight antigen (Fab) excess, caused maximum inhibition of the ADCC (80 to 90 percent) induced by the Hodgkin's IgG, when ^{51}Cr-labeled lymphocytes, with and without complexes, were incubated for 30 minutes at 37°C before being added to the ADCC system (*15*). The inhibition demonstrates that the cytotoxic effector cells bear Fc receptors. The target lymphocytes are, most probably, the TL populations, or subpopulations, since binding to purified BL populations did not occur.

It is of interest that spleens histologically uninvolved with Hodgkin's disease have increased Ig production (*8*) and are capable of producing antilymphocytic IgG. Hodgkin's disease lesions are, in many instances, difficult to recognize early on in their histological evolution (*16*). Splenic involvement may therefore be unrecognizable, by present techniques, during early stages of the disease. It is also possible that the immunological events accompanying the disease, at first local, may become increasingly reactive in other areas of the lymphoreticular system.

The present studies suggest that the circulating lymphopenia and deficient cell mediated immune responses often seen in Hodgkin's disease may be due to the production of autoantibodies directed against TL's or subpopulations of TL's. Two factors may contribute to the systemic compromise of TL immunocompetence. First, the amount of reactive IgG needed to produce ADCC is minimal in contrast to that required to bring about complement-dependent cytotoxicity (*13*), and second, autoantibody is produced in some apparently uninvolved tissues (spleen) as well as those tissues with histologically recognizable Hodgkin's lesions.

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