CYTOTOXIC IMMUNE CELLS WITH SPECIFICITY FOR DEFINED SOLUBLE ANTIGENS

IV. Antibody as Mediator of Specific Cytotoxicity*

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In an attempt to study model systems for allograft and tumor immunity, a number of in vitro cell-mediated cytotoxic systems have been investigated. Depending on the cytotoxic system studied, different types of cells, e.g. T lymphocytes, B lymphocytes, or macrophages, have been found to be involved in specific target cell destruction (see reference 1). Little is known about the parameters which determine the type of effector cell(s) that will function in a given experimental situation. Also, the mechanisms that lead to target cell destruction in the various systems and their possible in vivo interactions are poorly understood. In view of this complex situation, it seems desirable to start with the analysis of highly reproducible and well-defined systems.

In recent studies, we have investigated an in vitro cytotoxic system with specificity for defined soluble antigens. It has previously been found in an experimental autoimmune situation that red cells coated with soluble protein antigen can function as target cells for antigen specific cytolytic in vitro reactions (2). The present system consists of spleen cells from mice that have been immunized against conventional soluble antigens, such as heterologous proteins or hapten-carrier conjugates, and of ^{51}Cr-labeled target cells (chicken red blood cells, CRBC) to which the respective antigens had been covalently attached (3). Cell-fractionation studies (4, 5) have revealed that the lytic effector cell in this system is neither an antigen-specific T cell (as tested by helper function) nor an antigen-specific B cell (as tested by antibody-forming cell precursor function) and that it has distinct adherence properties (see reference 6). Greenberg et al. (7), working with antibody-coated CRBC and non-

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Abbreviations used in this paper: Ag-CRBC, antigen-coated CRBC; BGG, bovine γ-globulin; CRBC, chicken red blood cells; DB, dinitrophenylated bovine serum albumin; DM, dinitrophenylated mouse serum albumin; DNP, dinitrophenyl; HSA, human serum albumin; OA, ovalbumin; PBS, phosphate-buffered saline, pH 7.4; and SRBC, sheep red blood cells.

immune mouse spleen cells, demonstrated that in their system the effector cells were membrane-Ig negative as well as θ-antigen negative. These effector cells could be separated from other cells and were characterized as monocyte-like cells.[1]

The present paper reports investigations on the mechanism that leads to specific lysis of the antigen-coated target cells by immune spleen cells. Little is known about what confers specificity on monocyte- or macrophage-mediated cytotoxicity. Among the different factors that have been suggested are: specific macrophage-arming factor (SMAF, 8, 9), which might be a T cell product (9, 10), and cytophilic antibody (11, 12). Evidence will be presented that specific cytotoxicity in our system is mediated through specific antibody, which is produced by the immune cells, and which interacts with the target cell antigens through its combining sites. The interaction of antibody with the cytotoxic effector cells will be analyzed in the following report.[2]

Materials and Methods

The materials and methods used in the cytotoxic system have been described in detail previously (3–5) and therefore only a brief description will be given.

Mice and Immunization.—2–3-mo old CBA mice were used throughout the experiments. They were immunized subcutaneously with 0.1 ml antigen solution (1 mg/ml) emulsified in an equal volume of complete Freund's adjuvant (CFA).

Preparation of Antigen-Coated and ^{51}Cr-Labeled Target Cells.—0.1 ml of a 50% suspension of washed fresh CRBC was added to 3 ml of phosphate-buffered saline (PBS), pH 7.4, containing 35 mg albumin antigen. 0.35 ml of a solution of 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide-HCl (ECDI, 200 mg/ml) was added and the cells were incubated for 1 h at room temperature. They were then washed four times in PBS and tested for the presence of membrane-bound antigen using specific antisera and the passive hemagglutination microtest. For labeling with ^{51}Cr, about 10^7 Ag-CRBC in 0.2 ml PBS were incubated with 150 μCi $Na_2{}^{51}CrO_4$ (1 mCi/ml) for 3–4 h at room temperature. The labeled cells were washed twice with culture medium (Medium F13, containing 10% heat-inactivated fetal calf serum, penicillin, and streptomycin) and adjusted to a concentration of 10^5 cells/ml.

Test for Cell-Mediated Cytotoxicity.—The cytotoxic test was carried out in Wassermann plastic test tubes (11 × 55 mm), each of which contained 5×10^4 ^{51}Cr-labeled Ag-CRBC and varying amounts of immune or nonimmune spleen cells (see the corresponding ratio for each experiment) in a volume of 1 ml culture medium. Depending on the experiment, antibody or inhibitors were added to the incubation mixtures. Their final concentrations are indicated in each experiment. After incubation for about 18 h at 37°C in a 5% CO_2 atmosphere, the tubes were centrifuged and 0.5-ml aliquots of the supernatants removed. The radioactivity of the pellet and residual supernatant and the radioactivity of the 0.5-ml supernatant sample were measured in a well-type scintillation counter. After subtraction of the background, a release index (R.I.) was calculated:

$$\text{R.I.} = \frac{\text{radioactivity of the supernatant}}{\text{total radioactivity}} \times 100.$$

[1] A. H. Greenberg, L. Shen, and I. M. Roitt. 1973. Characterization of the antibody-dependent cytotoxic cell: a non-phagocytic monocyte? *Clin. Exp. Immunol.* **15:**251.

[2] V. Schirrmacher, B. Rubin, and H. Pross. 1973. Cytotoxic immune cells with specificity for defined soluble antigens. V. Analysis of the interaction of antibody with the cytotoxic effector cells in immune or nonimmune spleen cells. Manuscript submitted for publication.

The results are expressed as R.I. minus spontaneous release of target cells alone. Each value given is the mean of the figures obtained for three test tubes. The intragroup variance for a given experiment has been computed from the R.I. of all the test tubes. Use has been made of this variance to calculate the difference that would be significant ($P = 0.05$) in a student's t test between two experimental groups in the experiment.

Preparation and Use of Anti-Ig or Control Columns.—The preparation of glass bead columns coated with a polyvalent rabbit antimouse immunoglobulin serum (anti-Ig column) or with normal rabbit serum (control column) has been described (13). Sera or supernatants were absorbed on the columns by passing them through at room temperature and at a flow rate of about 1 ml/min. The eluates were concentrated to the original volume by negative pressure dialysis.

Separation of 19S and 7S Antibodies.—19S and 7S antibodies were separated by sucrose gradient centrifugation as described previously (14).

RESULTS

Absence of Carrier Specificiy.—The secondary anti-DNP response to dinitrophenylated mouse serum albumin (DM) in mice shows the phenomenon of carrier specificity (15), while the anti-DNP antibodies induced are hapten specific (16).[3] In order to evaluate the specificity characteristics of the cytotoxic reaction of DM-immune cells the role of the carrier molecule was investigated. The results in Table I show that DM-immune cells display the same degree of cytotoxicity to CRBC coated with dinitrophenylated bovine serum albumin (DB) as to CRBC-DM, while they are not cytotoxic to CRBC-OA. Since there is no cross-reactivity between the carrier determinants on DM and DB (16), it is concluded that the cytotoxic reaction of DM-immune cells is not carrier specific.

Inhibition of Cytotoxicity by Soluble Antigen or Hapten.—We further investigated the specificity of the cytotoxic system by inhibition experiments using as inhibitors free soluble antigen or hapten. Table II shows two such experiments. DM- and OA-immune cells were incubated in the presence or absence of either DNP-lysine (exp. I) or OA (exp. II) together with CRBC-DM, CRBC-DB, or CRBC-OA as target cells. Specific and complete inhibition was observed in both instances, and the existence of hapten-specific cytotoxicity is clearly demonstrated (exp. I).

The data in Table II show differences in the amount of OA or DNP-lysine that inhibited the respective cytotoxic system. On a molar basis, about a hundred times more DNP-lysine was needed to get the same degree of inhibition as with OA. When the serum antibodies of the OA- and DM-immune animals that were donating the immune cytotoxic cells were analyzed by passive hemagglutination inhibition, similar differences in the location of the inhibition curves were observed (Fig. 1). With increasing time after immunization both cytotoxicity inhibition curves and antibody inhibition curves shifted to lower antigen or hapten concentrations. From these data it is apparent that the cyto-

[3] See Discussion section concerning the immune response against new antigenic determinants.

TABLE I

Absence of Carrier Specificity of DM-Immune Cell Cytotoxicity

Target cell	S.R.*	Cytotoxicity‡ of immune cells§					
		Anti-DM			Anti-OA		
		150:1	75:1	25:1	150:1	75:1	25:1
CRBC-DM	7	35	26	20	5	9	7
CRBC-DB	8	37	28	23	9	9	10
CRBC-OA	7	6	5	7	37	30	23

* Spontaneous ^{51}Cr release (S.R.) from target cells incubated alone.

‡ Expressed as release index minus S.R.

§The immune spleen cells (3 wk) were incubated with the target cells at the indicated ratios; the smallest difference that would be significant ($P = 0.05$) in a t test between two figures was 4.6.

TABLE II

Inhibition of Immune Cell Cytotoxicity by Free Hapten or Antigen

Exp.	Target cell	S.R.*	Cytotoxicity‡ of immune cells§								Inhibitor‖
				Anti-DM				Anti-OA			
			F13	−8	−6	−4	F13	−8	−6	−4	DNP-lysine
I	CRBC-DM	5	42	37	16	8	4	—	3	3	
	CRBC-DB	8	39	39	21	9	3	—	—	—	
	CRBC-OA	6	6	5	2	3	49	—	40	42	
			F13	−9	−8	−7	F13	−9	−8	−7	OA
II	CRBC-DM	4	40	—	—	42	9	—	—	7	
	CRBC-OA	5	7	—	—	5	44	32	16	7	

* Spontaneous ^{51}Cr release (S.R.) of target cells incubated alone.

‡ Expressed as release index minus S.R.

§ Immune spleen cells, 4 wk (exp. I) or 2 wk (exp. II) after immunization with 100 μg DM or OA; the ratio of immune to target cells was 150:1. The smallest difference that would be significant ($P = 0.05$) in a t test between two figures was 3.5 (exp. I) and 5.5 (exp. II). —, not done.

‖ The inhibitors were present during the whole incubation period (18 h). The $\log_{10}$ molar inhibitor concentration in the final incubation mixture is indicated in the respective horizontal line. F13 means medium without inhibitor.

toxicity inhibition curves reflect the avidity or affinity (in the case of OA and DNP-lysine respectively) of the serum antibodies in the same animals.

Induction of Cytotoxicity in Nonimmune Spleen Cells by Target Cell-Bound Serum Antibodies.—Fig. 2 illustrates an experiment in which specific cytotoxicity was observed (*a*) with immune spleen cells, and (*b*) with immune

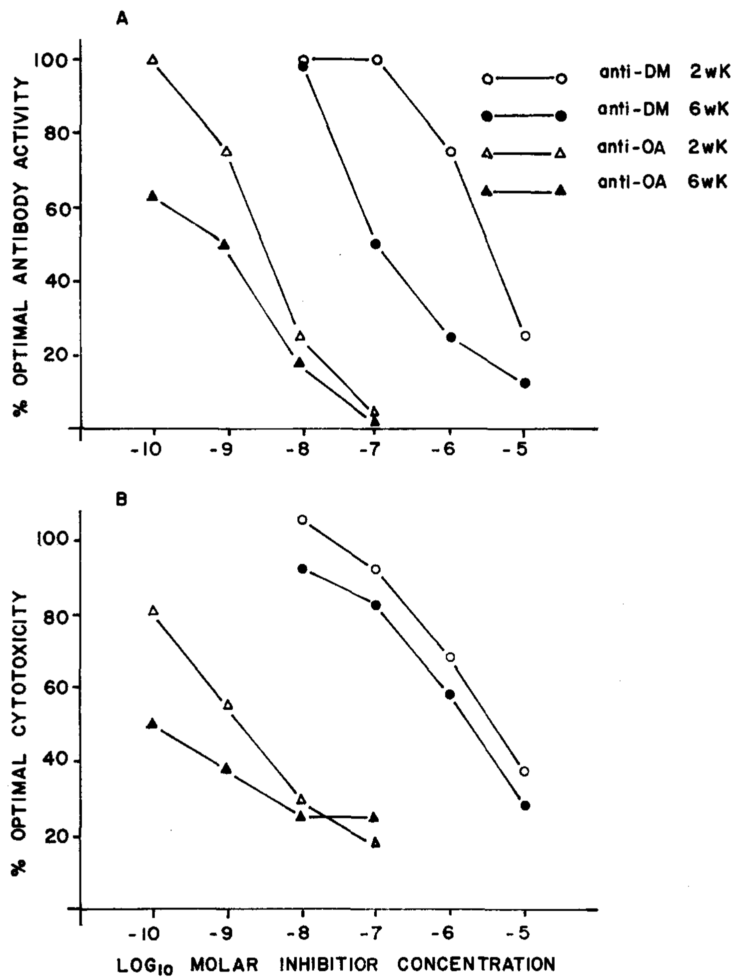

FIG. 1. Inhibition of antibody activity (*A*) and of immune cell cytotoxicity (*B*) by free soluble antigen or hapten. A Antibody activity in the sera from animals immunized 2 weeks (open symbols) or 6 weeks (closed symbols) before with 100 μg OA (triangles) or DM (circles) was determined by passive hemagglutination inhibition using SRBC-OA or CRBC-DM as indicator cells. The $\log_2$ titers for the pooled sera from three animals per group were: anti-OA (2 wk) 6.5; anti-OA (6 wk) 11.5; anti-DM (2 wk) 5.5; and anti-DM (6 wk) 5.5. Passive hemagglutination inhibition was carried out by titrating the sera in PBS containing OA or DNP-lysine respectively as inhibitors. B cytotoxicity of the immune spleen cells from the same animals was determined using ^{51}Cr-labeled CRBC-OA or CRBC-DM as target cells. The ratio of immune to target cells was 150:1. The specific cytotoxicity in the absence of OA or DNP-lysine as inhibitors was: anti-OA (2 wk) 28%; anti-OA (6 wk) 33%; anti-DM (2 wk) 57%; and anti-DM (6 wk) 49%. The smallest difference that would be significant ($P = 0.05$) in a t test between two points was 2.6.

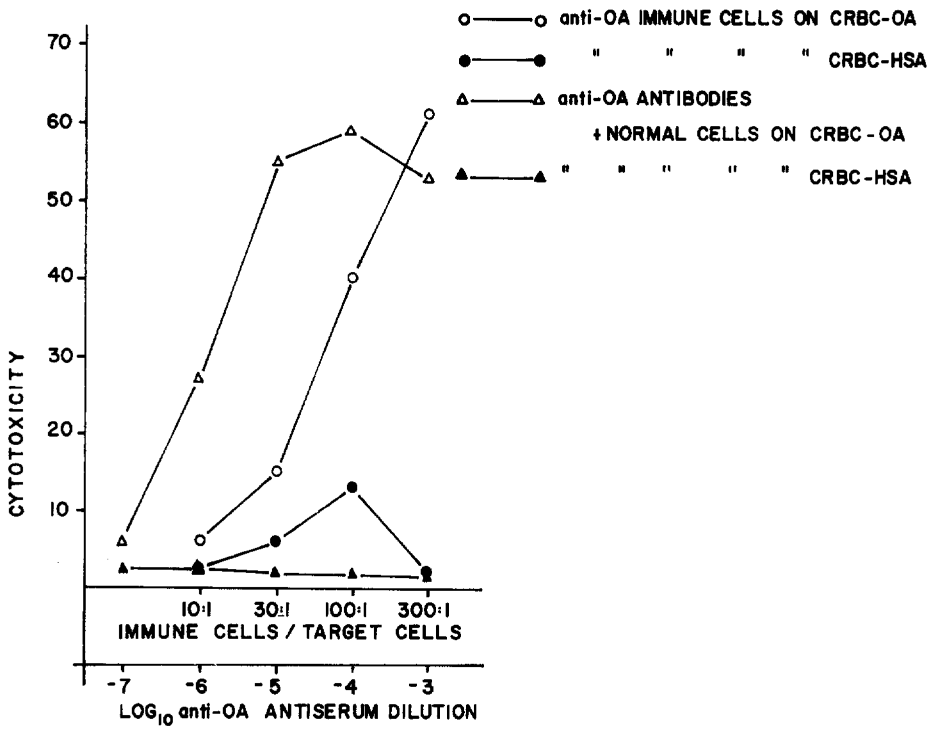

FIG. 2. Cytotoxicity of either OA-immune spleen cells (7 wk, circles) or of anti-OA antiserum from the same animals in presence of nonimmune spleen cells (= normal cells, triangles). The target cells were CRBC-OA (open symbols) and CRBC-HSA (solid symbols). The ratio of nonimmune spleen cells to target cells was 100:1. No specific cytotoxicity was observed with anti-OA antiserum at the indicated concentrations in the absence of nonimmune spleen cells. The smallest difference that would be significant ($P = 0.05$) in a t test between two points was 4.9.

serum from the same animals in the presence of nonimmune spleen cells. Cytotoxicity increased with increasing ratios of immune to target cells[4] as well as with increasing amounts of antiserum (starting at a dilution of 10^{-7}) at a constant ratio of nonimmune cells to target cells (100:1). While minute amounts of antiserum (10^{-4}–10^{-5} diluted) induced optimal cytotoxicity, higher concentrations ($\geqq 10^{-3}$) usually had a suppressive effect.

In order to characterize the nature of the cytotoxicity inducing factor in the immune serum, the antiserum was absorbed on an anti-Ig or on a control column. The results (Table III) show that anti-OA antiserum absorbed on an

[4] In earlier experiments (3) we observed a linear increase of cytotoxicity with increasing ratios of immune to target cells only up to a ratio of about 50:1. It was now possible to show that the suppression or relative reduction of cytotoxicity at higher ratios was due to the centrifugation of the tubes before the incubation period. This step, which increased the sensitivity of the system at low ratios, was omitted in the experiments reported in this paper.

TABLE III

Cytotoxicity of Nonimmune Spleen Cells in the Presence of Antiserum Absorbed on anti-Ig or Control Columns

Target cell	S.R.*	Cytotoxicity‡ of nonimmune spleen cells in presence of anti-OA antiserum§ which was					
		Unfractionated		Absorbed on an anti-Ig column		Absorbed on a control column	
		10^{-4}	10^{-6}	10^{-4}	10^{-6}	10^{-4}	10^{-6}
CRBC-OA	10	57	39	14	1	54	16
CRBC-HSA	10	0	0	1	1	3	0

* Spontaneous ^{51}Cr release (S.R.) from target cells incubated alone.

‡ Expressed as release index minus S.R.

§ Antiserum (inactivated 30 min at 56°C) from mice that had been immunized 6 mo before with 100 μg OA in Freund's complete adjuvant. Its $\log_2$ passive hemagglution titer with CRBC-OA was 9, after anti-Ig column absorbtion 1.5 and after control column absorbtion 8. The final dilution of the antiserum in the test is indicated. The ratio of spleen cells to target cells was 150:1. The smallest difference that would be significant ($P = 0.05$) in a t test between two figures was 4.1.

anti-Ig column was by a factor of about one hundred less active in inducing specific cytotoxicity in comparison with the same serum absorbed on a control column. These results indicate the immunoglobulin nature of the cytotoxicity inducing factor in the immune serum.

Induction of Cytotoxicity in Nonimmune Spleen Cells by Supernatants from Immune Cell Cultures.—The fact that serum antibodies bound to Ag-CRBC target cells induced cytotoxicity in nonimmune spleen cells does not necessarily mean that the present immune cell cytotoxic system functions according to the same principle, i.e. through the involvement of specific antibodies. In order to elucidate this point an attempt was made to directly demonstrate specific cytotoxicity inducing antibody activity in the supernatants of our immune cell cultures. Fig. 3 shows the positive outcome of such an experiment. DB-immune spleen cells were incubated in vitro under the conditions of the cytotoxic test, but in the absence of target cells, and the supernatants from fourteen such cultures were pooled. DNP specific cytotoxicity could be conferred on nonimmune spleen cells by these supernatants, as well as by their γ-globulin fractions, provided, of course, that DNP-coated target cells were added to the system to demonstrate the effect (Fig. 3 *A* and Fig. 3 *B*). Supernatants absorbed on an anti-Ig column were inactive (Fig. 3 *C*). It is thus shown that the immune spleen cells produce antibody in vitro in quantites sufficient to induce cytotoxicity in nonimmune spleen cells. Since we have also shown that the immune cell cytotoxic system has the specificity and affinity characteristics of serum antibodies (see above), we would conclude that cytotoxicity in our immune cell system is in fact mediated through specific antibodies.

Activity of Target Cell-Bound 19S and 7S Antibodies in the Induction of Cyto-

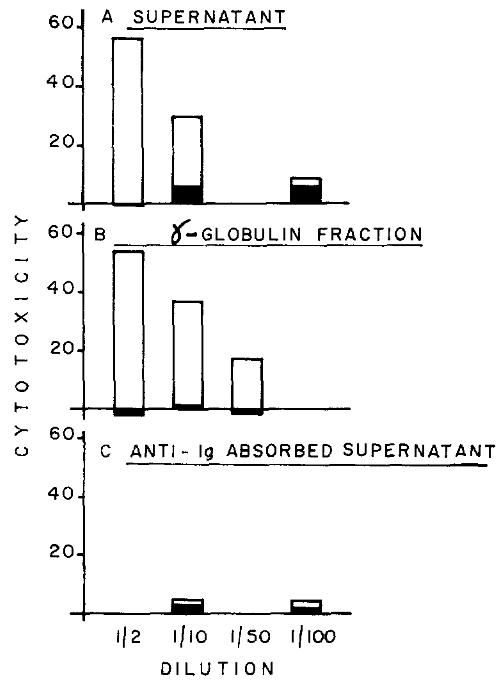

FIG. 3. Cytotoxicity of supernatants from DB-immune cell cultures in presence of nonimmune spleen cells and CRBC-DM (white columns) or uncoated CRBC (black columns) as target cells. The ratio of nonimmune spleen cells to target cells was 100:1. 4×10^7 DB-immune cells (4 wk) were incubated in 1 ml culture medium for 18 h at 37°C in a 5% CO_2 atmosphere. The supernatants of fourteen such cultures were pooled. The γ-globulin fraction was prepared from a sample (7 ml) by two successive precipitations with half saturated ammonium sulfate, with extensive dialysis against saline in between and afterwards. Another sample of the supernatant was absorbed on a glass bead column coated with rabbit anti-mouse Ig antiserum.

toxicity.—The next step in the analysis of the Ag-CRBC lytic system was to investigate whether the ability of antibodies to mediate specific cytotoxicity was restricted to a certain class (19S/7S) of antibodies. Fig. 4 shows the results of several experiments. 19S and 7S anti-DNP antisera which had the same passive hemagglutination titer were titrated in the presence of nonimmune spleen cells and CRBC-DM (white columns) or CRBC-OA (black columns) as target cells. While 19S antibodies from whole sera or from a 19S sucrose gradient fraction were virtually inactive in the cytotoxic test (Fig. 4 *A*), 7S antibodies from whole sera or from a 7S sucrose gradient fraction were very efficient in inducing cytotoxicity (Fig. 4 *B*).

DISCUSSION

Antibody-dependent cell-mediated cytolytic systems have been described in a number of instances, where antibody complexed target cells were incubated

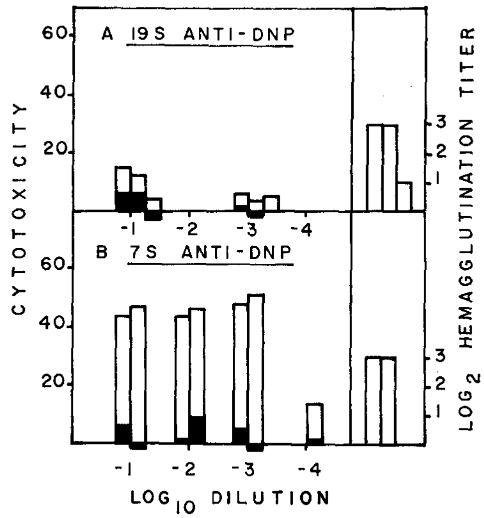

FIG. 4. Cytotoxicity of nonimmune spleen cells in presence of 19S (*A*) or 7S (*B*) anti-DNP antibodies and CRBC-DM (white columns) or CRBC-OA (black columns) as target cells. The ratio of spleen cells to target cells was 100:1. The DNP-specific hemagglutination titers of the different antibody fractions, indicated on the right part, were tested with DNP-HSA-SRBC. While the 19S antibody titers were sensitive to reduction with 0.2 M 2-mercapto-ethanol, the 7S titers were reduction resistant. 19S antisera were obtained from mice 9 days (first column) or 13 days (second and third column) after a single intraperitoneal injection of 100 μg DNP_{60}-BGG. The third column shows the activity of a 19S sucrose gradient fraction. 7S antisera were obtained from mice 6 wk (first column) or 6 mo (second column) after immunization with 100 μg DNP_{12}-BGG in Freund's complete adjuvant. The second column shows the activity of a 7S sucrose gradient fraction. All sera were inactivated 30 min at 56°C.

in vitro with nonimmune lymphoid cells. Mostly xenogeneic systems have been studied (17–19) but allogeneic (20, 21) and syngeneic (autoimmune, reference 22, and tumor immune reference 23) systems have been described as well. The cytolytic effector cells in these systems are believed not to belong to the T lymphocytes (see references 24 and 25). It has not yet been demonstrated if immune cell cytolytic systems can function according to the same principle, i.e. through a cooperation between an antibody-producing system and nonimmune effector cells. Synergistic and antagonistic effects of antibody on in vitro immune cell cytotoxicity have been described (26–28). The only safe conclusion that has been reached so far is that there exist in the immune system at least two alternative cell-mediated cytolytic pathways, one involving T lymphocytes (13, 29), the other non-T lymphoid or other cells (3, 31, 34). The exact nature of the latter effector cells is still under discussion. Non-T cell-mediated cytotoxicity has been attributed to any type of non-T cell-bearing membrane-bound F_c receptors: purified lymphocytes (18, 35), B cells (19, 25, 31, 36, 37); macrophages (11, 38–40); monocytes (41);[1] polymorphonuclear cells (42); and also to a new type of cell (43–45). Some of the studies that involved the use of anti-Ig sera (31, 36) for characterizing the effector cells might have been misleading, because inhibition of cytotoxicity might have reflected a blocking of F_c receptors via anti-Ig-Ig complexes rather than a direct effect on the effector cell surface structures (see references 4, 7, and 24).[2] Since the F_c receptors on lymphocytes and monocytes differ in their specificities for IgG subclasses (46, 47) aggregated myeloma proteins of different subclasses have been used in inhibition experiments in order to clarify whether the effector cells belong to the lymphocyte or to the monocyte lineage of cells. Unfortunately, different results have been obtained in different systems. The cytotoxic effector cells in the human peripheral blood showed subclass specificity characteristics of lymphocytes (45, 48), while in mouse spleen cells the cytotoxic effector cells shared the subclass specificities with the monocytes.[1] It therefore seems that depending on the cytotoxic system studied, different types of non-T cells may become activated by antibody-coated target cells to function as cytotoxic effector cells.

The present immune cell cytotoxicity system has certain advantages as compared with others: (*a*) it is generally applicable to the study of cytotoxic responses using any kind of soluble antigen (3),[5] (*b*) the target cell membrane is relatively stable (3), reducing the risk of production of inhibitory complexes; (*c*) the exact control of specificity is made possible by using the same type of target cell coated with different antigens; and (*d*) it is also possible to do fine specificity studies and even affinity or avidity analysis through inhibition by defined soluble antigens.

In the present study, three lines of evidence were obtained for the involvement of specific antibodies in the immune cell cytotoxicity system: (*a*) DM-immune cell cytotoxicity has been shown to be hapten specific (Tables I and II) and thus to follow the specificity characteristics of anti-DNP antibodies rather than of cellular immune responses (see reference 50). No specificity was found for the new antigenic determinants (NAD's, reference 16) introduced into the autologous protein by the hapten-coupling reaction. This is due to the fact that NAD's were not expressed on the CRBC-DM target cells, probably due to the carbodiimide-coupling reaction (V. Schirrmacher and B. Rubin, unpublished observations). (*b*) The cytotoxicity inhibition curves obtained in the presence of either free hapten (DNP-lysine in case of DM-immune cells) or soluble antigen (OA in case of OA-immune cells) reflected the affinity or avidity respectively of the serum antibodies from the same animals. Cytotoxicity ininhibition curves and antibody inhibition curves were at a similar position in both the DNP and the OA system, and both shifted with increasing time after immunization towards lower inhibitor concentrations (Fig. 1). (*c*) Supernatants from in vitro immune cell cultures contained an antigen-specific factor, which, like serum antibodies (Fig. 2 and Table III), induced cytotoxicity in nonimmune spleen cells when specific target cells were added. This supernatant ac-

[5] The system has already been successfully applied for studies on autoimmune thyroiditis (2, 49).

tivity was recovered in the γ-globulin fraction, and was absorbed on columns coated with polyvalent antimouse immunoglobulin serum (Fig. 3).

Thus, specificity is conferred on the effector cells in the immune cytolytic system by antibodies, which are produced during the incubation period of the test. We have no evidence for antibodies being brought into the system from the beginning, e.g. in form of cytophilic antibodies that were attached to cell surfaces (12), but we do know that cytotoxicity in the immune cell system starts only after a lag period of about 3 h, while in presence of antibodies it would start immediately.[2] Other evidence for the dependency of the immune cell cytotoxic system on active antibody synthesis in vitro comes from studies of MacLennan and Harding (51) who showed that their immune cell cytotoxic system was inhibited in presence of puromycin while the nonimmune cell system was not affected.

The ability of target cell-bound antibody to induce cytotoxicity in nonimmune spleen cells was restricted to the 7S antibody class (Fig. 4). Similar results have been obtained in other antibody-dependent cell-mediated cytotoxic systems (52, 53). It is difficult to explain why 19S antibodies were inactive in this cytolytic test, especially considering the fact that macrophages do possess a receptor for 19S antibodies (54).

SUMMARY

Spleen cells from mice immunized against ovalbumin (OA) or dinitrophenylated mouse serum albumin (DM) were found to be specifically cytotoxic in vitro towards target cells (chicken red blood cells) coated with these antigens. Inhibition of specific cytotoxicity was observed when free soluble antigen was added to the incubation mixtures. DM-immune cell cytotoxicity could be specifically and completely inhibited by DNP-lysine and was thus shown to be hapten specific. Complete and specific inhibition was also observed for OA-immune cell cytotoxicity using OA as inhibitor, but compared with the inhibition curves obtained with DNP-lysine, the OA cytotoxicity inhibition curves were shifted by a factor of about one hundred towards lower molar inhibitor concentrations. Very similar results were observed when the serum antibodies of DM- and OA-immune animals were analyzed by passive hemagglutination inhibition. With increasing time after immunization, both cytotoxicity inhibition curves and agglutination inhibition curves, shifted to lower antigen or hapten concentrations.

Specific cytotoxicity against antigen-coated target cells was induced in nonimmune spleen cells (*a*) by serum from immune animals, and (*b*) by supernatants from in vitro immune cell cultures. In both instances, the factor which induced antigen-specific cytotoxic activity could be absorbed on anti-mouse Ig columns, thus demonstrating its immunoglobulin nature. The ability of target cell bound antibodies to induce cytotoxicity in nonimmune spleen cells was restricted to the 7S antibody class.

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