STIMULATION OF ANTIBODY PRODUCTION TO THE HAPTEN 2,4-DINITROBENZENE BY AFFINITY LABELED MURINE LYMPHOID CELLS

I. Ability of Affinity Labeled Murine Lymphoid Cells to Activate the In Vivo Immune Response*

By DAVID A. LAWRENCE‡ and WILLIAM O. WEIGLE§

(From the Department of Experimental Pathology, Scripps Clinic and Research Foundation, La Jolla, California 92037)

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The interaction of T (thymus-derived) cells and B (bone marrow-derived) cells which results in an optimal humoral immune response has been well documented (1–3); however, the actual mechanisms involved in this cooperative process have not been clearly delineated. Numerous hypotheses for T- and B-cell interaction have been proposed (4, 5). Some reports suggest that T cells carry antigen to B cells, thus increasing antigen concentration and stimulating B cells (6); in addition, the T cells may further stimulate B cells through surface interaction (4). More recent reports suggest that T cells release both nonspecific (4, 7–9) and antigen-specific soluble factors (9) which may or may not be complexed with antigen; the antigen-specific T-cell factor may act as the first "signal" (10) and other T-cell products may act as the second signal for initiation of B-cell synthesis of antibody. In the case of T-cell factors, direct T- and B-cell interaction (cell "bridging" with antigen) would not be necessary. However, the posited mechanisms involved in T- and B-cell interaction may all function in varying degrees, depending on the characteristics of the antigen and the responding organisms.

In order to function in any direct cooperation with B cells, T cells must be capable of binding antigen. T cells have the ability to directly bind antigens (11–13). One such antigen which T cells have been shown to bind is 2,4-dinitrophenyl-human IgG (DNP-HGG)[1] (14); a binding specific, in part, for the DNP haptenic determinant. Other workers have also recently reported that T cells show specificity for haptenic deter-

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[1] *Abbreviations used in this paper:* Bm, bone marrow cells; Bm*, *m*-NBDF-labeled Bm cells; BSA, bovine serum albumin; BSS, balanced salt solution; DNP-HGG, 2,4-dinitrophenyl-human IgG; FCS, fetal calf serum; GRBC, goat red blood cells; *m*-NDBF, meta-nitrobenzenediazonium fluoborate; MP, murine IgA myeloma proteins; MRBC, mouse red blood cells; MRBC*, *m*-NBDF-labeled MRBC; NBm, normal Bm cells, NS, normal spleen cells; NT, normal T cells; S, spleen cells; S*, *m*-NBDF-labeled S cells, T*, *m*-NBDF-labeled T cells.

minants (15, 16). The direct binding of DNP-HGG by CBA/J thymus cells can be inhibited with the affinity labeling compound, meta-nitrobenzenediazonium fluoborate (*m*-NBDF), indicating that *m*-NBDF probably binds to an antigen-specific receptor of the T cell (14).

Since *m*-NBDF will covalently bind to DNP receptor active sites, it was decided to investigate its immunogenicity when coupled to syngeneic lymphoid cells. If an immune response developed, the only immunogen would be either coupled to or released from a specific type of lymphoid cell, thus making the study of the mechanisms involved in T-B-cell cooperation somewhat more straightforward.

In this investigation, the abilities of various combinations of lymphoid cells labeled with *m*-NBDF to produce an in vivo immune response to DNP were studied. The transfer of immunogenicity (into the irradiated recipients) by means of *m*-NBDF was shown to take place only if the T-cell or B-cell receptor was free to react with the *m*-NBDF on corresponding B or T cells, respectively. It was further shown that T cells are active participants in the response in that they do not just act as passive carriers of the haptenic determinant.

Materials and Methods

Animals.—The thymus, spleen (S), bone marrow (Bm), and mouse red blood cells (MRBC) were obtained from normal 6–8-wk old female CBA/J mice (Jackson Laboratories, Bar Harbor, Maine). Mice were maintained on Purina chow pellets (Ralston Purina Co., St. Louis, Mo.) and water with neomycin (1 mg/ml) ad libitum.

Preparation of Cells.—Spleens, thymuses, and tibias were removed from the mice after exsanguination; single cell suspensions were prepared as previously described (17). Briefly, thymuses or spleens were grated on stainless steel screens and then filtered through nylon netting. Bm cells were obtained by forcing media through the tibia marrows. A balanced salt solution (BSS) was used for the isolation of all cells, and all operations were done at 4°C. MRBC were obtained from the CBA/J mouse blood which was collected in a heparin-saline solution and washed with BSS.

Irradiation.—All mice to be irradiated were exposed to 900 R of γ-radiation from a ^{137}Cs irradiator at a dose rate of 123 rads/min. The irradiated, syngeneic recipient mice received cells within 3 h of irradiation. A dose of 3,000 R was used to irradiate cell suspensions; the cells were kept at 4°C during irradiation. Cell viability was not significantly changed when determined immediately after irradiation by trypan blue exclusion.

Antisera.—AKR anti-θ serum was prepared by the method of Reif and Allen (18), except that CE/J thymus cells were used; AKR anti-(CE/J) θ eliminates the possibility of any antibodies to the Ly determinants when used on CBA/J thymus cells. Cytotoxicity tests showed that the antiserum was specific for T lymphocytes. The anti-θ-treated S cells were obtained as previously described (14). The amplifying serum used for the hemolytic plaque assay was goat antimouse IgG serum.

Proteins.—Bovine serum albumin (BSA) was obtained from Armour & Co., Chicago, Ill., and human IgG (HGG) was obtained as Cohn fraction II through the courtesy of the American Red Cross National Fractionation Center, and was further purified by DEAE-cellulose column chromatography. DNP_{38}-BSA and DNP_{62}-HGG were prepared by following the method described by Eisen (19). Murine IgA myeloma proteins (MP) MOPC 315 (anti-DNP) and S121 were isolated from the serum of BALB/cSt female mice in which the MOPC 315 and S121 tumors, respectively, were implanted. DNP-HGG, MOPC 315 MP, and S121 MP

were labeled with ^{125}I (Cambridge Nuclear Co., Cambridge, Mass.) by a chloramine T procedure (20) in small volumes (21); the specific activities were 32, 15, and 24 μCi/μg, respectively.

Affinity Labeling of the Cell Preparations.—The DNP affinity labeling compound *m*-NBDF was prepared according to the method of Traylor and Singer (22). The *m*-NBDF concentration used for labeling was 5×10^{-5} M in BSS. Cell concentrations of lymphoid cell preparations and MRBC were 2×10^7 and 5×10^7, respectively; the labeling was performed for 2 h at 4°C. Lymphoid cell viability as determined by trypan blue exclusion was >75% before and after labeling.

Hemolytic Plaque Assay.—The number of antibody-producing cells was enumerated by a modification (23) of the Jerne plaque technique (24). Indirect plaques were determined using amplifying serum, goat antimouse IgG. BSA and DNP-BSA were covalently coupled to goat red blood cells (GRBC) and used as indicator cells.

In Vivo Measurement of the Immune Response to DNP.—Various doses of normal or treated cell types were injected intravenously into irradiated syngeneic mice and the number of plaque-forming cells (PFC)/10^6 S cells was measured at the time of maximum response (day 9) as determined by previous kinetic experiments. No additional immunogen other than labeled lymphoid cells was administered. The number of indirect plaques specific to DNP was calculated by subtracting the number of plaques to BSA-GRBC from the number of plaques to DNP-BSA-GRBC. Only indirect plaques were determined because the number of direct plaques was insignificant in comparison to the number of indirect plaques. The results were considered significant when $0.05 > P > 0.01$ by Student's *t* test.

Migration of Irradiated Affinity Labeled T Cells.—The *m*-NBDF-labeled T cells were labeled with ^{51}Cr by the method described by Sanderson (25). In addition, an aliquot was irradiated with 3,000 R. 20×10^6 of either irradiated or nonirradiated T cells were injected intravenously into the 900 R irradiated syngeneic mice, and migration was monitored by counting the radioactivity in various tissues of the recipient mice at 4, 24, and 48 h.

Anti-DNP Antibody Binding to m-NBDF-Labeled T Cells.—^{125}I-labeled MOPC 315 and S121 MP were incubated with 5×10^6 normal thymus cells or *m*-NBDF-labeled thymus cells in 0.1 ml of BSS for 30 min at 4°C. The cells were then suspended in 1 ml of BSS and centrifuged through 6 ml of 100% fetal calf serum (FCS). The cell pellet was resuspended in 1 ml of BSS and transferred to a smaller tube for counting. The cells were incubated with 20, 40, 80, or 200 ng of [^{125}I]MOPC 315 or [^{125}I]S121 MP.

Antigen Binding and In Vitro Incubation of S Cells.—An autoradiographic assay was employed to study S-cell binding of DNP-HGG and inhibition of this binding with *m*-NBDF (14). In vitro recovery from this inhibition was studied by incubating the S-cell preparations in vitro in accordance with the method of Mishell and Dutton (26). After 3, 8, 24, and 48 h of incubation, normal S-(NS) cell and *m*-NBDF-labeled S-cell cultures were removed, washed once in minimal essential media containing 5% FCS and 0.1% sodium azide, and examined for DNP-HGG binding by means of an autoradiographic assay.

RESULTS

In Vivo Synergism of Thymus and Bm Cells.—As shown in Table I, there was a slight but not significant increase in the number of PFC to DNP when *m*-NBDF-labeled T (T*) cells and normal Bm (NBm) cells were injected as compared to when normal T (NT) cells and NBm cells were injected into the irradiated, syngeneic recipients given no additional immunogen. This slight increase in the number of PFC to DNP did not occur after injection of T* cells with *m*-NBDF-labeled Bm (Bm*) cells. The Bm* cells plus NT cells did not show any enhancement of the immune response to DNP. NBm cells by them-

TABLE I

In Vivo Synergism of CBA/J Mouse Thymus and Bone Marrow Cells in the Immune Response to the DNP Group

Treatment*	Indirect PFC/10^6 spleen cells ± SE‡
NT + NBm	48 ± 6
T* + NBm	67 ± 8
T* + Bm*	39 ± 5
NT + Bm*	46 ± 7
NBm	20 ± 2
T*	12 ± 3

* Cells injected into syngeneic CBA/J recipients irradiated with 900 R. NBm, 3×10^7 normal bone marrow cells; NT, 10×10^7 normal thymus cells; Bm*, 3×10^7 *m*-NBDF-labeled bone marrow cells; and T*, 10×10^7 *m*-NBDF-labeled thymus cells.

‡ The results represent the averages from three or more separate experiments.

selves gave background levels of PFC to DNP similar to those previously reported for NS cells (27). The PFCs observed when T cells alone are injected are probably due to DNP specific B cells of the recipient which have escaped the effects of irradiation.

Ability of m-NBDF-Labeled Lymphoid Cells to Provide Stimulation for an In Vivo Immune Response to DNP.—Since there was a slight increase in the immune response to DNP when T* cells and NBm cells were injected into irradiated, syngeneic recipients, it was assumed that the response might be significantly enhanced if S cells were used intead of NBm cells. This assumption was based on the fact that spleen B cells seem to have a greater degree of immunological maturity than Bm cells (28). T* cells injected with NS cells into recipients with no additional immunogen produced a significant threefold increase in the number of PFC to DNP as compared to the number produced with NS cells alone (Table II). Twice as many T* cells (2×10^8) resulted in a significant four- to fivefold increase. In order to determine whether T cells in the NS population had any effect on enhancement, NS cells were treated with anti-θ serum and complement (C′). As shown, there was no significant change in number of PFC. When T* cells were injected in combination with *m*-NBDF-labeled S (S*) cells, there was no enhancement of the response; the number of PFC did not significantly differ from that obtained when S* cells alone were injected. This would seem to indicate that T* cells and S* cells do not interact. NT cells injected with NS cells gave a response even lower than the NS cell control. NT cells with S* cells gave a significant enhancement of the immune response to DNP indicating that there must be a direct T-B-cell contact in this system.

Irradiation of m-NBDF-Labeled T (T) Cells.*—The ability of the T* cells to produce an elevated in vivo immune response to DNP when injected with NS cells into irradiated, syngeneic recipients could be the result of either an active

TABLE II

In Vivo Ability of m-NBDF-Labeled CBA/J Mouse Thymus Cells to Initiate the PFC Response to the DNP Group

Treatment*	Indirect PFC/10^6 S cells ± SE‡
NS	55 ± 5
T* + NS	137 ± 22
2 × T* + NS	227 ± 37
T* + NS (anti-θ + C′)	144 ± 19
T* + S*	87 ± 15
S*	70 ± 4
NT + NS	37 ± 15
NT + S*	152 ± 33

* Cells injected into syngeneic CBA/J recipients irradiated with 900 R. NS, 3×10^7 NS cells; S*, 3×10^7 *m*-NBDF-labeled S cells; T*, 10×10^7 *m*-NBDF-labeled thymus cells; and NT, 10×10^7 normal thymus cells.

‡ The results represent the averages from three or more separate experiments.

involvement of T cells, such as the liberation of B-cell-stimulating products, or of a passive role, such as carrier of the affinity labeling haptenic determinant to the B cells. In an attempt to determine the function of T cells, they were irradiated with 3,000 R; a dose that would ordinarily destroy the normal functioning of all lymphoid cells since their mean lethal dose is approximately 520 R (29). The irradiation was performed either before or after affinity labeling of the T cells. As shown in Table III, there was no difference in number of PFC to DNP regardless of whether irradiation was given before or after labeling, but in both cases the immune response was inhibited by approximately 50%.

Migration of Irradiated Cells.—Since the immune response was reduced 50% after irradiation, it appears that some T cells may perform an active function in production of an immune response to DNP. There may be various subpopulations within the thymus cell preparation that function differently. The reduction, however, could be caused by a difference in the migration of irradiated cells, i.e., fewer of these cells may home to the spleen and therefore cause a reduction in the number of PFC/10^6 S cells. In order to determine if there was a difference in the migratory pattern, the cell preparations were labeled with ^{51}Cr and injected intravenously into irradiated recipients. There was no reduction in number of irradiated T* cells which homed to the spleen; in fact, by 48 h there were slightly more irradiated T* cells present in the spleen than non-irradiated T* cells (Table IV). Thus, spleen homing of T cells is apparently not affected by irradiation, but the ability of T cells to leave the spleen after reaching it may be affected by irradiation (30).

Cell Type Capable of Transferring the Immune Response to DNP.—As shown in Table II, T* cells in combination with NS cells will produce an enhanced immune response to DNP. S* cells alone are not capable of producing a significant increase in the number of PFC. In order to determine if a cell type

TABLE III

Effect of Irradiation on the In Vivo Helper Ability of the m-NBDF-Labeled Thymus Cells

Treatment*	Indirect PFC/10^6 S cells ± SE‡
NS	37 ± 6
T* + NS	185 ± 49
3,000 R-T* + NS	86 ± 11
T*-3,000 R + NS	99 ± 16

* Cells injected into syngeneic CBA/J recipients irradiated with 900 R. NS, 3×10^7 NS cells; T*, 20×10^7 *m*-NBDF-labeled thymus cells; 3,000 R-T*, 20×10^7 normal thymus cells irradiated with 3,000 R and then *m*-NBDF-labeled cells; T*-3,000 R, 20×10^7 *m*-NBDF-labeled thymus cells irradiated with 3,000 R.

‡ Each result represents the average from seven or more individual recipients.

TABLE IV

Ratio of the In Vivo Distribution of the m-NBDF-Labeled T (T) Cells to the 3,000 R Irradiated T* Cells*

^{51}Cr sample	Time after cell injection		
	4 h	24 h	48 h
Blood	0.64*	2.69	0.95
Lung	1.88	2.26	1.50
Thymus	1.00	1.00	0.92
Kidney	1.38	1.25	1.29
Spleen	1.04	0.83	0.79
Liver	0.97	1.05	1.07

* Represents (percent recovery of ^{51}Cr-nonirradiated T* cells)/(percent recovery of ^{51}Cr-irradiated T* cells); percent recovery equals cpm of ^{51}Cr T* cells per tissue/cpm of ^{51}Cr T* cells injected into 900 R irradiated, syngeneic CBA/J recipients. The ratios were obtained from three individual mice in each group.

other than T cells was capable of transfering the immune response to DNP (Table V), CBA/J MRBC and Bm cells were affinity labeled and transferred along with NS. MRBC* or Bm* cells plus NS did not significantly increase the number of PFC above that obtained with NS cells alone, whereas T* cells plus NS cells in this experiment gave a fourfold increase in number of PFC.

Anti-DNP Antibody Binding to m-NBDF-Labeled T Cells.—If T* cells can specifically stimulate DNP-specific B cells, then B-cell antigen-binding receptors must be able to recognize the haptenic determinant on the T cells. This possibility was investigated by attempting to determine whether anti-DNP antibody would preferentially bind to T* cells. It was assumed that the active site of an antibody molecule is similar to that of the cell receptor. MP 315 which has anti-DNP activity (31) and, as a control, the MP S121 were used so that cytophilic antibody properties would not be involved since it has recently been demonstrated that IgA molecules are not cytophilic for lymphocytes

(unpublished results). At all concentrations tested, T* cells always bound approximately twice as much 315 MP as the NT cells; there was no difference in the amount of S121 MP bound by T* and NT cells.

The ratio of the amount of 315 or S121 MP which was bound to T* cells as compared to NT cells is expressed as an average for all concentrations from three separate experiments ± SE. T*/NT for 315 MP was 2.14 ± 0.10 and T*/NT for S121 MP was 1.20 ± 0.09; these results were significantly different ($P < 0.001$) by Student's t test. Thus, it seems apparent that the haptenic determinant on T* cells can be recognized by DNP-specific antigen-binding sites of B-cell receptors.

In Vitro Turnover of DNP-HGG S-Cell Receptor.—As shown in Fig. 1, *m*-NBDF inhibition of DNP-HGG binding by S cells is gradually lost after in vitro incubation of the S cells. Recovery is faster from an initially lower concentration of *m*-NBDF (5×10^{-6} M), but even with 5×10^{-5} M *m*-NBDF

TABLE V

In Vivo Ability of m-NBDF-Labeled Bm, MRBC, and Thymus Cells to Initiate the PFC Response to the DNP Group

Treatment*	Indirect PFC/10^6 spleen cells ± SE‡
NS	55 ± 5
MRBC* + NS	62 ± 8
Bm* + NS	69 ± 9
T* + NS	204 ± 29

* Cells injected into syngeneic CBA/J recipients irradiated with 900 R. NS, 3×10^7 NS cells; MRBC*, 40×10^7 *m*-NBDF-labeled MRBC; Bm*, 5×10^7 *m*-NBDF-labeled Bm cells; and T*, 20×10^7 *m*-NBDF-labeled thymus cells.

‡ Each result represents the average of nine or more individual recipients.

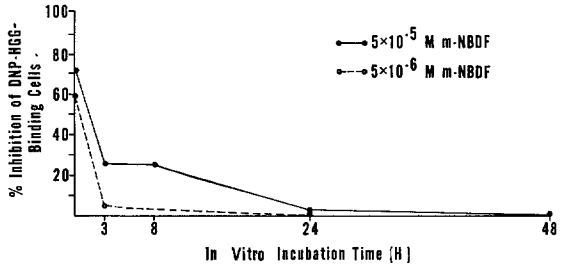

FIG. 1. In vitro recovery of mouse S cell binding of DNP-HGG after inhibition with *m*-NBDF. The ordinate represents the arithmetic mean from three separate experiments expressed as percent inhibition of DNP-HGG binding calculated from the following: 1 − (% *m*-NBDF-labeled S-cell binding of DNP-HGG/% unlabeled S-cell binding of DNP-HGG) × 100. The labeled and unlabeled S cells at each in vitro time were harvested from three separate cultures.

the inhibitory effect is completely gone by 48 h. The fact that in vitro recovery is not linear indicates that the cells are not in synchrony and/or not all cells are inhibited equally. The results also indicate that S cell receptors for DNP-HGG are released and replaced in 48 h or less; this is especially significant when considering the fact that T* cells plus NS cells gave an enhanced response to DNP, but T* cells plus S* cells did not significantly enhance the immune response to DNP.

DISCUSSION

The experiments reported here suggest that stimulation of B cells to produce antibodies to DNP may, in part, result from direct contact between T and B cells. The injection of either affinity labeled T (T*) cells and NS cells or NT cells and affinity labeled S (S*) cells into irradiated recipients that were given no additional immunogen resulted in an immune response to DNP; however, when T* and S* cells were injected, there was only a slight increase over the normal number of background PFC to DNP (27). Since there is an enhanced response to DNP with T* and NS cells or NT and S* cells, the simplest mechanism of interaction to account for this response would be cell to cell contact, although soluble factors probably also play a role (7–9). In the case of T* and NS cells eliciting an increased response, it is possible that an antigen-specific factor is liberated from the T* cells which in turn directly or indirectly stimulates the B cells; if direct cellular interaction is not involved, though, the response obtained by NT and S* cells would require that antigen be transferred from the B cells to T cells and back again through some intermediate system. The possible biological significance of such a complex system is difficult to comprehend when the simple mechanism of T- and B-cell interaction would suffice to explain the increased response. Interestingly, B* cells apparently can carry antigen to T cells and so initiate T-cell antigenic stimulation; a situation which is the reverse of what is usually proposed (6). The results of Rajewsky et al. (32) support the concept that efficient in vivo antibody induction relies on cell to cell interaction mediated by antigen bridging. The fact that T* and S* cells do not collaborate also favors the possibility of cell to cell contact, because when receptors are blocked there can be no bridging of cells and therefore no initiation of events for synthesis of antibodies to DNP. The absence of cell to cell interactions when T* and B* cell receptors are concomitantly blocked could similarly result when a great excess of antigen blocks receptors or passive antibodies block free determinants of an antigen already bound to one cell type. A previous study (14) has shown that *m*-NBDF specifically inhibits the binding of DNP protein conjugates by murine thymus and S cells, thus it is not unexpected that when *m*-NBDF is bound to T- and B-cell receptors it can block cell cooperation. Plotz (33) and Segal et al. (34) have used other affinity labeling compounds on lymphoid cells to inhibit a specific immune response to DNP or NIP. However, if T- or B-cell receptors are free to react with the covalently

bound *m*-NBD determinant on correspondingly labeled B or T cells, the *m*-NBD moiety can be immunogenic. de Weck (35) has shown that hapten conjugated to lymphoid cells is important to elicit a response, e.g., stimulation of lymphocytes by penicillin added to culture medium requires covalent binding of the pencillin molecule and that conjugation of penicillin to lymphoid cells is more stimulating for antigen-sensitive cells than conjugation to soluble proteins or to erythrocytes.

T and B cells apparently can "bridge" through the covalently bound haptenic determinant (the *m*-NBD residue) and only interact if one cell type is labeled. The capacity to bridge is evidenced by the ability of an anti-DNP antibody, MOPC 315, to specifically bind to affinity labeled T cells. This indicates that DNP-specific receptors on B cells could also bind to the *m*-NBD moiety on T cells assuming that the receptor and antibody active sites are similar. The bridging of T* and B or T and B* cells probably results from a specific receptor binding a *m*-NBD residue which has been bound to a membrane protein on the collaborating cell. However, an alternative possibility for bridging could be that the *m*-NBD moiety can be bound by the active site of a receptor and still be recognized by another receptor. Recognition, then, must be via different specificities of the *m*-NBD molecule. It has been shown that there is a heterogeneous population of anti-DNP antibodies (36), and receptors are probably also heterogeneous which may allow for mutual recognition of the *m*-NBD residue by two individual receptors. Even if the initial bridging is not due to specific receptors on T and B cells, T* cells still seem to preferentially stimulate B cells because T* cells can initiate a response to DNP; however, MRBC* and Bm* cells are incapable of eliciting a response with NS cells, even though a portion of the Bm cells is capable of being stimulated to synthesize antibodies. Therefore, the T* cells must contribute more to B-cell stimulation than just hapten presentation. After antigen bridging, T- and B-cell membranes may be attracted for further collaboration. The stimulation of the B cell through its receptors could then be due to a cross-linking of receptors by the T-cell membrane which provides the two postulated "signals" for antibody production (10). Direct cell contact would be advantageous even when considering T-cell factors (7–9); these factors may have a limited range, and cell contact might lower any diluting effects resulting from the cells not being in close contact. The in vitro system described by Basten and Feldmann (9) involving T- and B-cell separation with a membrane indicates that direct cell contact is not necessary; however, if the T and B cells were not separated by a membrane, the response might be further enhanced. The above discussion does not include the role of the macrophage. Many studies indicate that macrophages play an important intermediate role in the initiation of the humoral immune response (4, 9). Macrophages bridging T and B cells could easily fit into the mechanisms described. The membrane interactions would simply manifest themselves on or through the macrophage membrane.

The kinetics of S-cell blockage suggest that the first 24–48 h in the irradiated

recipient may be critical for the interaction of T and B cells, because when the T* and S* cells were injected there was no significant response to DNP, for the B-cell receptors are not free to react until 24–48 h after labeling. It is possible that when T* and S* cells are injected the response is delayed until the B-cell receptors are free to react with T* cells or their products, or that by the time B-cell receptors are free to react, the T* cells may have already shed their receptors, and cellular interaction may not be optimal. The in vitro receptor turnover was investigated by observing the recovery of DNP-HGG binding by the S* cells. The turnover time of surface immunoglobulins or receptors (37, 38) has been reported by others as being 6 h to 2 wk. The reason for this variation is not known. In the present study, the turnover time of the receptors which bind DNP-HGG was 24–48 h.

The helper function of T* cells observed in this investigation was not limited to ability to concentrate antigen and present it to B cells. In fact, although *m*-NBDF will selectively bind to the DNP-specific receptors on the lymphoid cells, it could also bind to other membrane proteins. In order to find out if the cells were only carriers of antigen and played no other functional role, T* cells were irradiated with 3,000 R, a dose which usually prevents DNA synthesis and eventually causes cell death (29). After irradiation of T* cells and injection along with NS cells into the recipients, the response to DNP was reduced but not completely ablated. Reduction of the response indicates that the T cells or at least a fraction of them were performing an active function other than that of carrier. Active involvement of T cells is confirmed by the result obtained when NT and S* cells were injected; there was an increase in number of anti-DNP PFC, and in this case, the T cells are not carriers but are actively involved in some other way. Miller et al. (39) using tolerant T cells coupled with antigen has given further evidence that T cells are more than just a passive carrier of the immunogen. A different in vivo migration of T* cells due to irradiation was experimentally ruled out as a possible cause for reduction in the response to DNP. The fact that irradiation apparently does not affect cell migration is in agreement with the result of Anderson et al. (30), who found no change in the S-cell homing of irradiated T cells. The 50% reduction in the number of PFC to DNP which results from irradiation of T* cells could be accounted for by there being: (*a*) subpopulations of active thymus cells varying in their sensitivity to irradiation; (*b*) thymus cells having two or more functions and these functions varying in their sensitivity to irradiation; or (*c*) a combination of the above, i.e., different types of T cells performing different functions and also varying in their sensitivity to irradiation.

The murine thymus has been shown to contain cells capable of binding DNP-HGG (14); some of these cells may have been naturally "activated" to DNP. This is not inconceivable since there are PFC cells in normal mice which produce antibodies to DNP (27). Thus, there may be T cells in various states of differentiation which have specificity for DNP, and these cells may have different sensitivities to the various effects of irradiation. T-cell factors may also

be responsible for maintenance of a lowered immune response because the "allogenic" or nonspecific factor can still be released from irradiated T cells and stimulate B cells (8). Subpopulations of T cells do reside in the thymus since, for example, there are cortisone-resistant and -sensitive populations (40). Subpopulations of T cells have been described and are referred to as T_1 and T_2 cells (41). These subpopulations of T cells have different functional properties and different radiosensitivities (42). Feldmann and Basten (43) have shown that the helper effect of NT cells and primed T cells is reduced with mitomycin C, but activated T cells are not affected. These results with mitomycin C may be similar to irradiation effects since both are inhibitors of DNA synthesis and cell division. Other studies have shown that the effects of irradiation can inhibit completely or only slightly inhibit the helper activity of T cells (30, 44–46). Whether or not T cells are radiosensitive or radioresistant is in itself controversial. Their radiosensitivity may be determined by their immune status, i.e., whether they have been or are being stimulated by antigen and/or are differentiating. The reduction in the response to DNP due to irradiation of T cells may result from loss of direct T- and B-cell contact which again points out the possible advantage of membrane interactions. Radiation effects are known to cause membrane changes which result in cell leakage (47, 48). Membrane deformities may interfere with T- and B-cell interaction and lower, if not ablate, the efficiency of the interaction.

SUMMARY

The ability of meta-nitrobenzenediazonium fluoborate (*m*-NBDF)-labeled thymus and spleen (S) cells to transfer immunity to 2,4-dinitrophenyl (DNP) into irradiated syngeneic recipients was investigated. There was a significant increase in the number of anti-DNP plaque-forming cells (PFC) when *m*-NBDF-labeled thymus cells and normal spleen cells, or normal thymus cells and *m*-NBDF-labeled spleen cells were transferred, but not when both thymus- and S-cell populations were labeled and injected together into irradiated recipients. The ability of these cell populations to cooperate and enhance the in vivo immune response to DNP is discussed. The T cells seem to be actively involved in the development of this response; they participate beyond the mere role of carrying and presenting antigen to the B cells. It is suggested that cell to cell contact between T and B cells may be an important factor in the elicitation of an immune response. In addition, the cellular interaction is affected by irradiating the thymus cell preparation and the initiating interaction required for antibody synthesis probably occurs within 48 h after injecting the cell populations into the syngeneic irradiated recipients.

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