DELETION OF HAPTEN-BINDING CELLS BY A HIGHLY RADIOACTIVE $^{125}$I CONJUGATE*

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Immunocompetent cells capable of recognizing antigens most likely possess antigen-binding receptors on their membranes and hence are called antigen-binding cells (ABC) (1-5). Ada and Byrt (6) provided a direct approach to the study of ABC by showing that exposure of lymphoid cells to radioactive flagellin specifically abolished the immune response to that antigen. Their technique is termed antigen-induced "suicide" because the flagellin-binding cells are "killed" by the highly radioactive antigen which adheres to them. We now report that lymphoid cells recognizing dinitrophenyl (DNP), referred to as DNP-binding cells, can be inactivated specifically by highly radioactive DNP-protein conjugates in which the radioactivity is not part of the hapten, but is solely confined to the carrier moiety. Moreover, the results show that the recognition of DNP by lymphoid cells is independent of the carrier to which DNP is bound.

Materials and Methods

Animals.—6-7-wk old male (C57BL/6 X DBA)F1 (hereafter referred to as BDF1) were obtained from Jackson Laboratory, Bar Harbor, Maine.

Haptens.—2,4-Dinitrophenyl sulfonic acid, twice recrystallized, (Eastman Kodak Co., Rochester, N.Y.) and picryl sulfonic acid (2,4,6-trinitrobenzene sulfonic acid), twice recrystallized, (Nutritional Biochemicals Corporation, Cleveland, Ohio) were used.

Synthetic Antigens.—$\Sigma$-DNP-lysine ($\Sigma$-DNP-Lys) was a gift from Dr. Frank Richard.

Protein Carriers.—Keyhole limpet hemocyanin (KLH) (Pacific Biomarine Supply Co., Venice, Calif.); human gamma globulin (HGG) (Pentex Biochemical, Kankakee, Ill.); bovine serum albumin (BSA) (Armour Pharmaceutical Co., Chicago, Ill.); and isogenic mouse gamma globulin (MGG) were prepared as described elsewhere (7).

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Abbreviations used in this paper: ABC, antigen-binding cells; BDF1, (C57BL/6 X DBA)F1; BSA, bovine serum albumin; CFA, complete Freund’s adjuvant; DNP, dinitrophenyl; $\Sigma$-DNP-Lys, $\Sigma$-DNP-lysine; HGG, human gamma globulin; KLH, keyhole limpet hemocyanin; MGG, isogenic mouse gamma globulin; PFC, plaque-forming cells; SRBC, sheep red blood cells; TNP, trinitrophenyl.

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**Hapten-Protein Carrier Conjugates.**—The following conjugates were used (after preparation as previously described [7]): DNP$_{48}$-KLH, DNP$_{62}$-KLH, DNP$_{46}$-KLH, DNP$_{21}$-BSA, DNP$_{65}$-HGG, DNP$_{10}$-MGG, and TNP$_{10}$-KLH.

**Iodination of Conjugated or Unconjugated Protein Carriers.**—Iodination with carrier-free $^{125}$I (New England Nuclear Corp., Boston, Mass.; catalogue No. NEZ-033) was done by the chloramine-T method (8), as modified by Unanue (9). The specific activity and degree of substitution of each preparation will be detailed in the results.

**X-Irradiation.**—Recipient mice were irradiated with 960 R using a Westinghouse deep therapy unit (Westinghouse Electric Corp., Pittsburgh, Pa.) (250 kv, 15 mA, target distance 50 cm). The mice were placed in a Lucite container which was divided into 12 cells. This was rotated on a turntable to insure a uniform exposure to the X-rays.

**Spleen Cell Suspensions.**—Spleens were excised, pooled, and minced with scissors. The fragments were gently pressed through a tantalum gauze and suspended (50 $\times$ 10$^6$ cells/ml) in a 10% fetal calf serum in minimal essential Eagle's medium (Microbiological Associates, Inc., Bethesda, Md.) supplemented with glutamine, penicillin, and streptomycin in appropriate concentration, after being washed twice.

**Experimental Design.**—Spleen cells of unprimed mice were incubated in vitro on a rotating table with unlabeled or $^{125}$I-labeled antigen at 0°C for 2 hr (in two separate experiments the exposure times were 30 min and 23 hr, respectively). The concentration of the antigen was 1 $\mu$g of protein/50 $\times$ 10$^6$ spleen cells per ml of medium. The cells were then washed twice with the same medium and collected by centrifugation at low speed at 4°C, and their viability checked by the trypan blue exclusion test (a viability of 95% was the usual result). The radioactivity of the cells recovered in the centrifuged pellet was 0.2–0.5% of the original amount. 50 $\times$ 10$^6$ of the treated cells (resuspended in 0.2–0.3 ml of medium) were then injected intravenously into lethally irradiated syngeneic recipient mice.

**Immunization.**—1 hr after the adoptive transfer, recipients were immunized with 1 mg of DNP-KLH (DNP$_{62}$-KLH or DNP$_{46}$-KLH) given intraperitoneally (i.p.) in complete Freund's adjuvant (CFA), or, in some instances, with 1 mg of TNP-KLH or 0.1 ml of 20% sheep red blood cells (SRBC, Colorado Serum Co., Denver, Colo.) in saline.

**Hemolytic Plaque Assay.**—8 days after immunization, the mice were sacrificed and their splenic direct plaque-forming cells (PFC) were enumerated by Rittenberg's modification (10) of Jerne's hemolytic plaque assay (11) in which trinitrophenyl (TNP)-coated SRBC are used to detect the anti-DNP response. TNP is used because conjugation of DNP to SRBC is, for technical reasons, unreliable. The antibody detected by this method is actually anti-DNP that cross-reacts with TNP; for the sake of brevity, the PFC detected by this method will be referred to as anti-DNP PFC. In the case of mice immunized with TNP-KLH, the same target cells were used, whereas, SRBC were used as target cells when the anti-SRBC response was tested. In certain instances, indirect PFC were revealed by a rabbit anti-mouse IgG antiserum (12).

**Statistical Analysis.**—Statistical analysis was done according to Student's $t$ test. For PFC, the geometric mean for each group was calculated.

## RESULTS

**Kinetics of Inactivation of the DNP-Binding Cells.**—In preliminary experiments, it was found that incubation of spleen cells with $^{125}$I-labeled antigen for 30 min resulted in a significant diminution of their immune response to DNP. Increasing the incubation for 2 hr further decreased the immune response, when compared with controls incubated with unlabeled DNP-KLH. However, a 23 hr incubation had no further influence (Fig. 1). Therefore, most subse-
sequent experiments were carried out using an in vitro exposure time to the radioactive antigen of 2 hr.

Specific Deletion of the Anti-DNP Response.—Spleen cells of unprimed male BDF₁ mice were incubated in vitro on a rotating table with unlabeled or ¹²⁵I-labeled DNP₄₈-KLH (5 μCi/10⁶ spleen cells; moles of iodide/moles of KLH ranged from 90 to 100; specific activity ranged from 232 to 250 μCi/μg) at 0°C for 2 hr. Cells incubated with DNP-KLH-¹²⁵I were unable to mount a response in the irradiated host to DNP. The suppression involved both direct and indirect PFC to DNP, indicating that both 19S and 7S antibody formation depend on the presence of DNP-binding cells. Whether one or two subpopulations of hapten-binding cells mediate both immune responses is unknown. The suppression was antigen specific because recipients of cells treated with DNP-KLH-¹²⁵I were able to respond normally to an unrelated antigen, namely SRBC (Table I).

Are the "Natural" or Background Anti-DNP PFC the Hapten-Binding Cells?—To test this, two experiments were carried out. In one, direct PFC were assayed 24 hr after in vitro incubation of unprimed BDF₁ spleen cell suspensions, for 2 or 24 hr, with DNP-KLH-¹²⁵I. In the other, the influence of incubating spleen cells with the radioactive conjugate was tested in an adoptive transfer system. In neither experiment did the treatment diminish the number of anti-DNP
PFC (Table II). Thus, the natural anti-DNP PFC are not affected by the highly radioactive antigen, and, therefore, they are not the DNP-binding cells required for the immune response to DNP-KLH. The observation, in

**TABLE I**

| No. of mice | In vitro pretreatment of cells | Immunization* | PFC/spleen (± se)† |
|-------------|--------------------------------|---------------|-------------------|
|             |                                | Direct (SE)   | Indirect (SE)     |
| 6           | DNP_{48}-KLH                   | DNP_{48}-KLH  | 22,751 (4977)     |
| 6           | DNP_{48}-KLH-125I              | "             | 3624 (1539)§      |
| 8           | DNP_{48}-KLH                   | SRBC          | 4603 (1109)       |
| 9           | DNP_{48}-KLH-125I              | "             | 3589 (1101) \|    |

* Immunized with 1.0 mg of DNP_{48}-KLH in CFA i.p. or 0.1 ml of 20% SRBC in saline i.p.

† Geometrical mean of anti-DNP PFC or anti-SRBC PFC/spleen (± se).

§ DNP-KLH-125I-treated group differed significantly from the control group (0.001 < P < 0.01 for direct anti-DNP PFC and 0.05 < P < 0.1 for indirect anti-DNP PFC).

|| Direct anti-SRBC PFC did not differ significantly in groups treated with DNP_{48}-KLH-125I or DNP_{48}-KLH. The low number of anti-SRBC PFC is explained by the fact that the assay was done 8 days after immunization, i.e., 3 days after the peak of the response to SRBC.

**TABLE II**

| Pretreatment of spleen cells | PFC/10⁶ spleen cells at 24 hr | PFC/10⁶ spleen cells (± se)§ at 8 days after adoptive transfer |
|-----------------------------|--------------------------------|---------------------------------------------------------------|
|                            | SRBC PFC                      | Corrected DNP PFC                                             |
|                            | DNP PFC                       |                                                               |
| DNP-KLH                    | 0.3                           | 2.6                                                          |
| DNP-KLH-125I (2 hr)        | 0.3                           | 2.3                                                          |
| DNP-KLH-125I (24 hr)       | 0.3                           | 3.4                                                          |

* DNP_{48}-KLH-125I specific activity 234 μCi/μg antigen; 5 μCi/10⁶ spleen cells; 0.02 μg/10⁶ spleen cells.

† Background anti-DNP PFC corrected by subtraction of background anti-SRBC PFC.

§ Lethally irradiated mice (four to eight per group) were repopulated with 50 X 10⁶ spleen cells from unprimed donors. These spleen cells had been incubated in vitro for 2 hr with DNP_{48}-KLH or DNP_{48}-KLH-125I. 8 days later the background PFC to DNP and SRBC were assayed. Data are the geometrical mean (± se) of each group.

\| There was no significant difference between anti-DNP PFC in groups treated with unlabeled or with 125I-labeled DNP KLH.

nonimmunized animals, that most of the lymphoid cells which bind SRBC (the rosette-forming cells) are not the background PFC to SRBC supports this conclusion (13).

**Hapten Specificity of Inactivation.**—This was determined in the next set of experiments. First, DNP-KLH-125I-treated spleen cells were used to repopu-
late two groups of lethally irradiated recipient mice, one of which was challenged with DNP-KLH and the other with TNP-KLH (Table III). The PFC producing anti-DNP cross reactive with TNP (elicited by DNP-KLH) were suppressed (group 3), but the PFC elicited by immunization with TNP-KLH were unaffected (group 4). Second, it should be noted that hapten-binding cells can be deleted in a noncross-reacting system; i.e., incubation with TNP-KLH-\(^{125}\)I abolished the immune response to TNP-KLH as tested with TNP-SRBC target cells (group 7). Third, incubation of spleen cells with TNP-KLH-\(^{125}\)I resulted in suppression of the DNP response as tested by TNP-SRBC (group 5). Judging by the relative amounts of radioactivity required to abolish responses to DNP or TNP, some DNP-binding cells seem to be more radio-sensitive than those responding to TNP only (group 6).

**Lack of Carrier Specificity in the Deletion of DNP-Binding Cells by Different DNP-\(^{125}\)I Conjugates.**—We next determined whether inactivation of DNP-binding cells by the radioactive conjugate was carrier specific; i.e. if the immune response to the hapten is suppressed only when the conjugate used to inactivate the spleen cells in vitro, and the one employed to challenge the recipient animals in vivo, are the same. Three different \(^{125}\)I-labeled conjugates of DNP (aside from DNP-KLH-\(^{125}\)I) were used to treat spleen cells in vitro. The X-irradiated recipients of these cells were then immunized with DNP-

**TABLE III**

**Hapten Specificity of Suppression by Treatment with DNP-KLH-\(^{125}\)I or TNP-KLH-\(^{125}\)I**

| Group | Antigen | \(\mu\)Ci/\(\mu\)g antigen | Immunization | PFC/spleen (\(\pm\) se) |
|-------|---------|-------------------------|--------------|------------------|
| 1     | DNP-48-KLH | --                      | DNP-62-KLH  | 19,087 (3133)    |
| 2     | TNP-110-KLH | --                      | TNP-110-KLH  | 33,437 (9182)    |
| 3     | DNP-48-KLH-\(^{125}\)I | 100 5 | DNP-62-KLH  | 2254 (859)§      |
| 4     | --        | 193 5                   | TNP-110-KLH  | 29,047 (7877)\||
| 5     | --        | 293 5                   | DNP-62-KLH  | 1004 (509)§      |
| 6     | --        | 194 9                   | TNP-110-KLH  | 33,225 (8085)\||
| 7     | TNP-110-KLH-\(^{125}\)I | 293 25 | TNP-110-KLH  | 5496 (1220)§      |

* 1 mg of antigen DNP-KLH or TNP-KLH in CFA i.p.

\(\mu\) Data represent the geometrical mean of anti-DNP PFC or anti-TNP PFC of each group (five to eight per group) (\(\pm\) se).

§ Experimental groups when compared with control mice immunized with DNP-62-KLH differed significantly (\(P < 0.001\)).

\| Not significantly different when compared with control mice immunized with TNP-110-KLH.

¶ Experimental group when compared with control mice immunized with TNP-110-KLH differed significantly (\(P < 0.001\)).
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KLH. The experimental design was similar to the preceding, except for the differences in conjugates. In all instances, the direct anti-DNP PFC were suppressed (Fig. 2). By contrast, no decrease (the increased numbers of PFC shown in Fig. 2 are not significant) in anti-DNP PFC occurred when the cor-

![Graph](image-url)  
**Fig. 2.** Absence of carrier specificity by different DNP-carrier protein-$^{125}$I conjugates. The hatched column represents the geometrical mean of the number of direct PFC (± se) in a group of 10 control mice repopulated with spleen cells treated with nonradioactive DNP$_{48}$-KLH. The different white columns represent the geometrical mean of the number of direct anti-DNP PFC (± se) of groups of mice (6-12 per group) repopulated with spleen cells treated with different $^{125}$I-labeled antigens, i.e., DNP$_{48}$-KLH, DNP$_{10}$-MGG, DNP$_{35}$-HGG, DNP$_{21}$-BSA. In all instances, the radioactivity was 5 μCi/10$^6$ spleen cells. Specificity activity of DNP$_{48}$-KLH, 250 μCi/μg; of DNP$_{10}$-MGG, 190 μCi/μg; of DNP$_{35}$-HGG, 244 μCi/μg; of DNP$_{21}$-BSA, 135 μCi/μg; of KLH, 355 μCi/μg; of MGG, 248 μCi/μg; of HGG, 290 μCi/μg; of BSA, 366 μCi/μg. Moles of iodide/moles of carrier: of DNP$_{48}$-KLH, 60; of DNP$_{10}$-MGG, 12; of DNP$_{35}$-HGG, 14; of DNP$_{21}$-BSA, 5; of KLH, 100; of MGG, 15; of HGG, 18; of BSA, 12. The lower shaded area represents the number of direct anti-DNP PFC (± se) in mice immunized with 1 mg of KLH in CFA i.p. 1 hr after X-irradiation with 960 R and repopulated with 50 $\times$ 10$^6$ spleen cells from unprimed BDF1 donors 6-wk old. The immune response to DNP was significantly suppressed in all the different experimental groups treated with $^{125}$I-labeled DNP conjugates ($P < 0.001$); whereas, in the groups treated with the different nonconjugated radioactive carriers, although the response was increased, no significant difference was found between each group and the control group. Vertical bar represents one se.
responding highly radioactive proteins were used in the nonconjugated form. This was the case even when KLH-^{125}I was used. However, in view of a recent report by Unanue (9) of the specific inactivation of KLH-binding cells by KLH-^{125}I, another experiment was done (Table IV), with five times the amount of radioactivity and conditions similar to his. In this case, about 50% reduction of anti-DNP PFC was obtained with 25 μCi KLH-^{125}I/10^6 spleen cells, whereas about 80% reduction was obtained when only 5 μCi DNP-KLH-^{125}I/10^6 spleen cells were used.

**Specific Inhibition by DNP-Protein Conjugates of the Deletion of Anti-DNP Response.**—Since suppression of the anti-DNP response of spleen cells was

| No. of mice | In vitro pretreatment of cells | Immunization* | PFC/spleen (± se) |
|-------------|--------------------------------|---------------|------------------|
|             | Antigen |μC/μg antigen |μC/10^6 cells | DNP-BSA-^{125}I |
| 6 | KLH | — | — | DNP-BSA-^{125}I | 36,260 (3956) |
| 6 | KLH-^{125}I | 330 | 25 | DNP-BSA-^{125}I | 16,977 (1494) § |

* 1 mg of DNP-BSA-KLH in CFA i.p.
† Geometrical mean of direct anti-DNP PFC (± se).
§ KLH-^{125}I-treated group differed (0.001 < P < 0.01) from KLH-treated control group.

independent of the ^{125}I-labeled protein carrier to which DNP was attached during the in vitro incubation, we next determined whether the inhibition of such a deletion was also carrier independent. Groups of mice (five mice per group) were repopulated after lethal X-irradiation with spleen cells treated in vitro before their exposure to ^{125}I-labeled DNP conjugates (DNP-MGG-^{125}I, DNP-BSA-^{125}I), to unlabeled DNP-KLH, KLH or Σ-DNP-Lys. The immune response to DNP was preserved when DNP-KLH (25 μg/10^6 cells) was used to inhibit the inactivation by nonhomologous DNP-protein carrier-^{125}I conjugates (antigen concentration 0.02–0.03 μg/10^6); however, KLH (under similar conditions) had no such effect, thus demonstrating the specificity of the inhibition (Table V). This result is a further indication that DNP-binding cells recognize and bind the hapten regardless of the carrier to which it is bound. The unsuccessful inhibition by Σ-DNP-Lys, even under a tenfold increased molar concentration, may be explained by the possibility that this monovalent ligand binds less efficiently to its specific receptor on the surface of a lymphoid cell and was removed from its specific attachment when the spleen cells were washed.

**Duration of Specific Deletion of Anti-DNP Response.**—Incubation of normal mouse spleen cells with DNP-BSA-^{125}I resulted in a suppression of their response to DNP after in vivo stimulation with DNP-KLH in the irradiated host (Fig. 2). The duration of the deletion of anti-DNP response was tested in groups of mice immunized with DNP-KLH at different time intervals after
the adoptive transfer, and their anti-DNP PFC enumerated 8 days after
immunization (namely 1, 2, and 3 wk after the cell transfer). The results indicate
that both direct and in direct anti-DNP PFC were suppressed for 2 wk; by
the 3rd wk the immune response was normal (Fig. 3).

Factors Determining the Inactivation.—In the course of these experiments, it
became apparent that three technical factors were important in inactivating

| TABLE V |
| Specific Inhibition of the Deletion of Anti-DNP Response |

| Group | In vitro pretreatment with unlabeled antigen* | In vitro incubation with DNP-125I conjugate† | PFC/spleen (± sE)§ |
|-------|---------------------------------------------|-----------------------------------------------|--------------------|
| 1     | —                                           | —                                             | 22,228 (1941)      |
| 2     | —                                           | DNP-MGG-125I                                  | 6816 (599)         |
| 3     | —                                           | DNP-BSA-125I                                  | 5117 (551)         |
| 4     | DNP-KLH                                     | DNP-MGG-125I                                  | 18,527 (3002)      |
| 5     | DNP-KLH                                     | DNP-BSA-125I                                  | 37,165 (11,173)    |
| 6     | KLH                                         | DNP-BSA-125I                                  | 1447 (320)§        |
| 7     | Σ-DNP-Lys                                    | DNP-BSA-125I                                  | 4921 (535)¶        |

* Unprimed spleen cell suspensions were prepared as described in Materials and Methods. Before their exposure to DNP-125I conjugate, they were incubated for 1 hr at 0°C on a rotating table with either DNP-KLH (3 x 10⁻¹¹ m/10⁶ cells), KLH (3 x 10⁻¹¹ m/10⁶ cells), or Σ-DNP-Lys (3 x 10⁻¹⁰ m/10⁶ cells), and thereafter washed twice in minimal essential Eagle’s medium, their viability checked, and their concentration readjusted to 50 x 10⁶/ml.

† 125I-labeled DNP-MGG (2 x 10⁻¹⁰ m/10⁶ cells; 10 μCi/10⁶ cells; specific activity 300 μCi/μg antigen). 125I-labeled DNP11-BSA (3 x 10⁻¹⁰ m/10⁶ cells; 10 μCi/10⁶ cells; specific activity 400-440 μCi/μg).

§ Geometric mean (± sE) of direct anti-DNP PFC in groups of mice (five mice per group) repopulated with 50 x 10⁶ spleen cells after lethal X-irradiation. All were immunized 1 hr after transfer of cells with 1 mg of DNP-KLH in CFA i.p., and 8 days later their immune response to DNP was assayed.

¶ Results show no statistical significant difference between group Nos. 4 and 5 and untreated controls (group No. 1); whereas, they were significantly different when compared with radioactive-treated controls (P < 0.001) (group Nos. 2 and 3).

‖ Group Nos. 6 and 7 differed (P < 0.001) from the untreated controls (group No. 1); whereas, they were not significantly different from the radioactive-treated controls (group No. 3).

DNP-binding cells: (a) the specific radioactivity of the conjugate (which relates directly to the degree of iodide substitution); (b) the concentration of antigen per cell; (c) the total amount of radioactivity per cell, which is directly related to the product of the two previous factors. The data in Table VI illustrate these observations for DNP-KLH-125I. There was no effect when low specific radioactivity and high antigen concentration were used, and only partial suppression was achieved when a condition of high specific radioactivity and low antigen concentration was present. In addition, despite a constant
Fig. 3. Duration of specific deletion of anti-DNP response. Black columns represent the number of direct PFC (± se) and the white columns the number of indirect PFC (± se) of groups of mice (five mice per group) repopulated with spleen cells treated in vitro with unlabeled or 125I-labelled DNP21-BSA (0.01 μg/10^6 spleen cells; 4 μCi/10^6 spleen cells; specific activity 123 μCi/μg antigen). All animals were immunized 1 hr after transfer with 1 mg of DNP6-KLH in CFA i.p. The columns on the extreme left represent the direct and indirect PFC respectively of controls transplanted with cells treated in vitro with unlabeled DNP21-BSA (the results were pooled since there was no significant statistical difference in the number of PFC in controls assayed 1, 2, or 3 wk after adoptive transfer of cells). The lower shaded area represents the number of anti-DNP PFC (± se) in mice immunized with 1 mg of KLH in CFA i.p. 1 hr after X-irradiation with 960 R and repopulated with 50 X 10^6 normal spleen cells. The results differ significantly (P < 0.001) from controls in experimental groups assayed 1 or 2 wk after transfer of cells; whereas there was no significant difference in the experimental group assayed after 3 wk. Vertical bar represent one se.

### TABLE VI

| μCi/μg DNP6-KLH | No. of DNP-KLH molecules X 10^6/10^6 cells | μCi/10^6 cells | PFC/spleen (± se) |
|-----------------|-----------------------------|----------------|-----------------|
| 66              | 0.56                        | 0.5            | 9343 (1910)     |
| 80              | 1.30                        | 1.4            | 9502 (3564)     |
| 100             | 3.33                        | 5.0            | 8183 (2799)     |
| 250             | 1.84                        | 5.0            | 2254 (859)*     |
| 450             | 0.83                        | 5.0            | 857 (259)*      |
| 82              | 4.51                        | 5.0            | 3467 (1021)*    |
| 64              | 11.60                       | 10.0           | 7181 (2350)     |

The various experimental conditions are listed on the first three columns. The fourth column represents the direct anti-DNP PFC/spleen obtained in lethally irradiated recipient animals (four to eight mice per group) repopulated with 50 X 10^6 untreated or treated spleen cells with DNP-KLH-125I, after challenge with 0.2 mg of DNP6-KLH in CFA i.p. * These three groups are significantly different from the controls. In addition, they are significantly different among themselves as determined by “Studentized Range Q,” a more conservative version of the “Least Significant Difference” (LSD).
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different ABC by highly radioactive antigens also demonstrate that specific requirements for each antigen exist, since the conditions under which ABC were inactivated, were spread over a wide range of the above mentioned parameters. Whether the difference in the degree of inactivation by highly radioactive hapten-carrier conjugates (Tables III and IV) reflect a difference in the radiosensitivity of various populations of ABC, or whether it is due to a difference in the number of cells recognizing a given antigenic determinant, is yet unknown. It is also possible that differences in the binding affinities of the highly radioactive conjugates for the receptors on the antigen-binding cells account for these results.

DISCUSSION

The results show that in vitro exposure of normal mouse spleen cells to a highly radioactive labeled DNP-protein carrier conjugate, in which the radioactivity was not part of the hapten but solely confined to the carrier moiety, inactivated specifically the cells able to form 19S (direct PFC) and 7S (indirect PFC) antibodies to the same hapten. Furthermore, the inactivation of DNP-binding cells was highly specific for the hapten and independent of the carrier to which the hapten was bound. Although the technique of deleting ABC by in vitro exposure to radioactive antigen was originally called “antigen-induced suicide,” it could just as likely constitute a “murder.” Regardless of which terminology is chosen, there is no direct evidence that the ABC are indeed killed by this method. The specific suppression of the immune response could as well be explained by the inactivation of ABC by an amount of radiation injury that only prevents cell proliferation. This deletion was transient (2–3 wk). It may suggest that either new DNP-binding cells developed during the random maturation of lymphoid stem cells in the inoculum transplanted into the irradiated host, or that the original DNP-binding cells were not killed but only temporarily inactivated, and had recovered from the irradiation injury during the 3 wk interval. In a recent publication, Unanue (16) also reported that the immune response to KLH (in mice transplanted with bone marrow cells treated in vitro before transfer with KLH-\textsuperscript{125}I) was only temporarily suppressed.

How can we account for the finding that the immune response to a hapten was prevented by treatment with a hapten-protein-\textsuperscript{125}I conjugate? Three possibilities can be considered: (a) carrier-binding cells were inactivated; (b) DNP-binding cells were deleted; and (c) a combination of both (a and b) occurred. The possibility that cells binding antigenic determinants of the carrier were inactivated cannot be ruled out, but is not likely because: (a) In vitro treatment of cells with KLH-\textsuperscript{125}I had no effect on the DNP response, whereas treatment of cells under similar conditions with DNP-KLH-\textsuperscript{125}I markedly suppressed the DNP response (Fig. 2); (b) The response to DNP was suppressed regardless of which radioactive carrier conjugate the lymphoid cells
were exposed to in vitro. We presume that DNP-binding cells were specifically inactivated by the radioactivity confined to the carrier moiety, which was brought into the immediate environment of the cell by union of DNP with its specific receptor on the surface of the hapten-binding cell. Therefore, to inactivate a lymphoid cell recognizing a given antigenic determinant, the radioactivity does not need to be part of this determinant.

It is worth noting that the specificity of the receptor of the hapten-binding cell closely mimics the specificity of the anti-DNP antibody, since in both instances, they bind DNP independently of the carrier to which it is attached. Absence of carrier specificity in hapten-binding cells was further demonstrated in the experiments in which inhibition of inactivation was performed. Suppression of the DNP response was prevented when cells were pretreated in vitro with unlabeled DNP-KLH before their exposure to highly $^{125}$I-labeled nonhomologous DNP conjugates (Table V). Lack of carrier recognition by hapten-binding cells was also shown by others when immunoadsorbent columns were used which depleted hapten-binding cells (DNP, NIP), regardless of the carrier attached to the immunoadsorbent (17, 18).

The immune response to DNP after in vitro exposure of spleen cells to KLH-$^{125}$I was either normal or only partially suppressed (Fig. 2, Table IV). Diminution of the anti-DNP response occurred only when the total amount of radioactivity of KLH-$^{125}$I was increased fivefold (as compared with previous experiments using DNP-KLH-$^{125}$I). This observation raises several possibilities: (a) Spleen cells engaged in helper function are less radiosensitive; (b) Helper cells are exposed to less radiation damage due to fewer receptors on their surfaces (19); (c) Helper cells can still fulfill their function in spite of radiation injury that prevents only their proliferation and not their antigen-binding capacity; (d) The number of helper cells in the spleen is in such an excess, that, even after inactivation, enough cells remain to fulfill this function. Our data do not permit choosing from any of these possibilities. However, they are in agreement with the observation of Roelants and Askonas (20), who showed that although in vitro exposure of hemocyanin-primed spleen cells to hemocyanin-$^{125}$I suppressed antibody formation to this antigen after challenge in vivo, this treatment did not interfere with the anti-hapten response to DNP-hemocyanin. They attributed this and their other findings to an excess of hemocyanin-specific helper cell activity in the hemocyanin-primed spleen cells.

The apparent lack of carrier recognition by hapten-binding cells raises the question of the role of carrier recognition in the various immune phenomena. For example, it is interesting to note that one of the carriers used in these experiments (as a highly $^{125}$I-labeled DNP conjugate) was isogeneic MGG, which was shown to be the most effective carrier to induce tolerance of DNP (7). Further study of this model should uncover more aspects of the cellular basis of antigen recognition and its importance in the development of both tolerance and immunity.
SUMMARY

Exposure of normal mouse spleen cells in vitro to highly 125I-labeled dinitrophenyl (DNP)-protein carrier conjugates specifically inactivated cells able to mount an immune response to that hapten after in vivo challenge. The deletion was hapten specific and independent of the radioactive carrier to which the hapten was bound. DNP-binding cells were inactivated by radioactivity that was not part of the hapten, but was solely confined to the carrier moiety. The deletion of the anti-DNP response lasted 2-3 wk and could be specifically inhibited.

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