

2,4-DINITROPHENYL RECEPTORS ON MOUSE THYMUS AND SPLEEN CELLS*

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It has been demonstrated that bone marrow-derived lymphoid cells, or B cells,¹ and thymus-derived lymphoid cells, or T cells, have antigen-specific receptors on their plasma membranes. Direct evidence for this has been provided for the B cell population with techniques such as autoradiography (1-3) and immunoadsorbent depletion of specific lymphoid cells (4, 5), whereas it has been more difficult to obtain direct evidence that T cells bind antigen. Most of the data on antigen-binding cells in the thymus have been obtained by indirect methods such as antigen suicide (6) and inhibition of T cell functions by anti-immunoglobulin sera (7-9). In recent studies, however, antigen-binding T cells have been detected by a rosette formation assay (10), enzymatic methods (11, 12), and autoradiography (13). Since it does appear that the specific receptors are most likely immunoglobulin in nature, it is not surprising that immunoglobulins have been easily detected on the surfaces of B cells (14-16), whereas their presence on T cells has been more difficult to demonstrate (17, 18).

In order to further characterize antigen-specific receptors, especially those on T cells, the present investigation was undertaken to study the 2,4-dinitrophenyl (DNP) receptors on spleen and thymus cells. These specific receptors were investigated because of some interesting aspects of the immune response to DNP which include (a) the presence of agglutinating anti-DNP antibodies in the sera of nonimmunized humans (19); (b) the existence of a number of myeloma proteins of mice and man with anti-DNP activity (20); and (c) the occurrence of a large number of antibody-forming cells to DNP in normal and

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¹ *Abbreviations used in this paper:* B cells, bone marrow-derived cells; BSS, balanced salt solution; DNP, 2,4-dinitrophenyl; FCS, fetal calf serum; HEPES, *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid; HGG, human IgG; MEM, minimum essential medium; *m*-NBDF, meta-nitrobenzenediazonium fluoborate; *p*-NBDF, para-nitrobenzenediazonium fluoborate; OA, ovalbumin; T cells, thymus-derived cells.

immunized animals (21, 22). It could, therefore, be expected that a large number of lymphoid cells with DNP receptors may be detectable. Autoradiography has been employed to quantitate the antigen-binding lymphocytes in thymuses and spleens of normal CBA/J mice and to examine the characteristics of the receptors with antisera specific for immunoglobulin chains and fragments. In these studies we have detected qualitative differences between thymic and splenic DNP-binding receptors.

Materials and Methods

Thymus and Spleen Cell Preparation.—The thymus and spleen cells were obtained from normal 5–7-wk-old CBA/J mice (Jackson Laboratory, Bar Harbor, Me.). Spleens and thymuses were removed from the mice after exsanguination; cell suspensions were prepared as described by Davie and Paul (3).

Antigens and Iodination.—Human IgG (HGG) was obtained as Cohn fraction II from American Red Cross and was further purified by DEAE-cellulose column chromatography; ovalbumin (OA) was obtained from Pentex Biochemical, Kankakee, Ill., and bovine serum albumin (BSA) was obtained from Armour & Co., Chicago, Ill. The DNP-HGG, DNP-BSA, and DNP-OA were made following the method described by Eisen (23); the conjugates had 55–60, 30–40, and 13–15 mol DNP/mol protein, respectively. The proteins and conjugates were labeled with ^{125}I (Cambridge Nuclear Co., Cambridge, Mass.) by a chloramine-T procedure (24) in small volumes (25). The DNP-Lys-Iyr (Cyclo Chemical Co., Los Angeles, Calif.) was ^{125}I -labeled by the method of Revoltella et al. (26). The specific activity of the different preparations varied from 35 to 70 $\mu\text{Ci}/\mu\text{g}$.

Antisera.—Antimouse immunoglobulin sera were obtained from rabbits or goats that had been injected with either normal mouse IgG or mouse myeloma proteins incorporated into incomplete Freund's adjuvant. The mouse immunoglobulins were isolated from the sera by Pevikon electrophoresis followed by Sephadex G-200 exclusion chromatography. Fab and Fc fragments were isolated by DEAE-cellulose chromatography after brief digestion with papain as previously described (27). The antisera were made specific for the IgG and IgA Fc fragments by absorption with Fab fragments and κ -light chains (MOPC 46). Since it was not possible to obtain Fab and Fc fragments from mouse IgM, either by papain or trypsin digestion,² the antiserum specific for IgM was prepared by injecting rabbits with μ -chain. The μ -chains were isolated from reduced and alkylated IgM by Sephadex G-100 exclusion chromatography in 1 N acetic acid. The following proteins were used for immunization: normal mouse IgG and IgG Fab fragments, κ -chain MOPC 46, myeloma proteins S-121 (IgA), and MOPC 104E (IgM). All antisera were heat inactivated for 30 min at 56°C and absorbed twice with $1/5$ vol of packed CBA/J erythrocytes. Anti- θ serum was prepared by the method of Reif and Allen (28) and shown by cytotoxicity tests to be specific for T lymphocytes.

Affinity-Labeling Compounds.—The DNP affinity-labeling compounds used were para-nitrobenzenediazonium fluoborate (*p*-NBDF) and meta-nitrobenzenediazonium fluoborate (*m*-NBDF). The *p*-NBDF (Eastman Organic Chemicals Co., Rochester, N.Y.) was recrystallized two times from methanol. The *m*-NBDF was prepared according to the method described by Traylor and Singer (29).

Autoradiographic Assay.—The autoradiographic method employed was a slight modification of that described by Davie and Paul (3). 20×10^6 lymphoid cells were suspended in 0.2 ml of minimal essential medium (MEM) which contained 0.1% sodium azide and 10% heat-inactivated fetal calf serum (FCS); 150–200 ng of ^{125}I -labeled antigen was added. The cell smears were made on microscope slides cleaned with methanol. Autoradiographic exposure

² Spiegelberg, H. L. Unpublished data.

time was 7–8 days. The cells were then stained with Giemsa and examined for silver grains. The criterion used for determining a positive binding cell was at least 10 grains on or surrounding the surface of a morphologically intact, small lymphocyte; 5000–10,000 cells per slide were counted. Each experiment was repeated three or more times with individual spleen or thymus cell preparations.

Inhibition of Antigen-Binding Cells.—Inhibition experiments were performed with unlabeled DNP-HGG, DNP-BSA, and HGG by preincubating each with a sample of the lymphoid cell suspension for 30 min on ice before the addition of the ^{125}I -labeled antigen. For the antisera inhibition studies, cells were preincubated for 2 h in MEM with *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (HEPES) buffer (Flow Laboratories, Inglewood, Calif.), which contained 0.1% sodium azide and 10% heat-inactivated FCS, and the cells were not washed before the addition of the ^{125}I -labeled antigen. A 2 h preincubation in balanced salt solution (BSS) followed by a wash was employed, however, for most of the inhibition studies with DNP-Lys, *p*-NBDF, and *m*-NBDF. In all inhibition experiments, control preparations of each cell pool were included and incubated without inhibitors. Each value represents three or more separate experiments.

Treatment of T Cells with Anti- θ Antiserum.—Samples of pooled normal thymus cell suspensions (200×10^6 cells) were incubated with heat-inactivated AKR anti- θ serum or normal AKR serum, 1:10, for 45 min at 37°C and after centrifugation for another 45 min at 37°C with guinea pig serum, 1:5, which was previously absorbed with agarose (50 mg/ml) (30) and CBA/J erythrocytes in order to remove specific and nonspecific cytotoxicity. After this treatment, the cells were layered on 100% FCS and the large cell clumps were allowed to settle out. The cell suspension was then removed and centrifuged twice through FCS; the final cell pellet was resuspended and tested for antigen binding.

Cortisone Treatment.—Mice were injected intraperitoneally with 2.5 mg of cortisone acetate (Merck, Sharp & Dohme, West Point, Pa.) and their thymuses were examined 48 h later (31). Cells from thymuses of five mice, which were treated in the above manner, were pooled so that 20×10^6 cells could consistently be used for the autoradiographic assay.

RESULTS

Antigen-Binding Cells in Normal Mice.—The percentage of normal CBA/J spleen cells which bound DNP-HGG (0.99%) was much greater than that binding either the hapten, DNP-Lys-Tyr (0.09%), or the carrier, HGG (0.23%) (Table I). The same was true for the percentages of the antigen-binding T cells. The percentage of DNP-HGG-binding cells in the thymuses (0.15%) was approximately $\frac{1}{6}$ of that in the spleens, and there was a greater degree of variance in the percentage of DNP-HGG-binding cells detectable in the thy-

TABLE I
Percentage of Antigen-Binding Lymphoid Cells of Normal CBA/J Mice

Antigen*	Spleen	Thymus
DNP ₅₇ -HGG	0.99 ± 0.16‡	0.15 ± 0.07
DNP ₁₄ -OA	0.19 ± 0.07	<0.02
DNP-Lys-Tyr	0.09 ± 0.03	<0.02
HGG	0.23 ± 0.04	<0.02

* Antigens labeled with ^{125}I .

‡ Standard deviation.

muses (0.08–0.22%) than was observed in the spleens (0.84–1.2%). In order to study another DNP conjugate with fewer DNP residues, DNP-OA was employed. The number of antigen-binding cells was different when the two DNP conjugates, DNP₁₄-OA and DNP₅₇-HGG, were compared; fewer spleen cells bound DNP-OA (0.19%) than DNP-HGG (0.99%), and no thymus cells binding DNP-OA were detected.

Effect of Anti- θ Treatment on the Number of Antigen-Binding T Cells.—In order to determine if the antigen-binding cells detected in the thymus preparations could be immigrant B cells, the T cell population in these preparations was reduced by treatment with anti- θ serum and complement before the addition of the labeled DNP-HGG. If the binding cells in the thymus preparations were B cells, this treatment should have increased the percentage of antigen-binding cells. However, as shown in Table II, no such increase was observed as

TABLE II
Effect of Anti- θ Serum on DNP Binding by Normal CBA/J Thymus Cells

Experiment	Treatment	T cell death†	Ag-binding cells
		%	%
1*	Normal AKR serum	—	0.12
	AKR anti- θ	50	0.12
2	Normal AKR serum	—	0.22
	AKR anti- θ	80	0.13
3	Normal AKR serum	—	0.11
	AKR anti- θ	68	0.12

* A sample of 200×10^6 cells from a thymic cell pool were used for treatment with the normal and anti- θ sera.

† Determined by cell counts and cell viability assayed by the exclusion of trypan blue.

compared to treatment of the same thymus cell preparation with normal AKR serum and complement. A 50 and 68% reduction of T cells produced no significant difference in the number of DNP-HGG-binding cells, and after an 80% reduction of T cells, there was still no increase; in fact, the number of cells binding DNP-HGG was reduced.

Effect of Cortisone Treatment on the Number of DNP-HGG-Binding T Cells.—CBA/J mice were injected with cortisone before their thymus cells were harvested in order to determine if the thymic cells which bind DNP-HGG belong to the cortisone-sensitive or cortisone-resistant T cell population. As can be seen in Table III, there was no significant difference in the number of DNP-HGG-binding cells in the thymus cell preparations from normal and cortisone-treated mice. As judged by the number of grains seen over the binding cells, it was shown, however, that about one-fifth of the cells in the cortisone-treated

populations bound larger amounts of DNP-HGG than the T cells from the normal mice.

Specificity of DNP-HGG Binding by Normal Spleen and Thymus Cells.—In order to demonstrate the specificity of the DNP-HGG binding by the spleen and thymus cells, the inhibitory effects of various compounds were tested. Table IV shows that the binding of DNP-HGG by spleen and thymus cells could be inhibited by the DNP conjugates but not by the carrier protein, HGG, even at a concentration 100 times that of the DNP conjugates. The hapten, DNP-Lys, was also used for inhibitory studies. Significant differences between the spleen and thymus cell binding of DNP-HGG could be detected when DNP-Lys was employed under different conditions. As shown in Table V, DNP-Lys significantly inhibited both the thymus and spleen cell binding of DNP-HGG if it was not removed before the addition of the ^{125}I -labeled DNP-HGG.

TABLE III
Effect of Cortisone Treatment on the Number of DNP-HGG-Binding Cells in Mice Thymuses

Thymus cells	Positive binding cells/ 10^4 cells		Ag-binding cells
	<15 grains/cell	>15 grains/cell	
			%
Normal*	9	--	
	14	—	0.12 \pm 0.03‡
	12	---	
Cortisone treated§	11	3	
	10	3	
	8	4	0.17 \pm 0.05
	20	4	
	16	5	

* Untreated 6-wk-old CBA/J mice.

‡ Standard deviation.

§ Each group represents a thymic cell pool of five mice which had been treated with 2.5 mg of cortisone acetate 48 h before thymus removal.

TABLE IV
Inhibition of [^{125}I]DNP-HGG Binding in Normal CBA/J Mouse Lymphoid Cells

Inhibitor*	Concentration	Percent inhibition	
		Spleen	Thymus
	$\mu\text{g/ml}$		
DNP ₃₅ -BSA	10	80 \pm 7‡	>88
DNP ₅₇ -HGG	10	81 \pm 5	>88
HGG	1,000	0	0

* 30 min preincubation with cells and present with labeled antigen.

‡ Standard deviation.

However, if the cells were only preincubated with DNP-Lys, the hapten did not significantly inhibit the binding of the DNP-HGG by the T cells (7% inhibition), whereas 57% of the spleen cell binding was still inhibited. This suggests that the T cells have a lower avidity for the DNP hapten than a portion of the cells in the spleen cell preparations.

The DNP affinity-labeling compounds, *m*-NBDF and *p*-NBDF, which have been shown to bind covalently to the combining site of antibodies to DNP (32, 33), were used to further study the specificity of the receptors and inhibition of their binding of DNP-HGG. The DNP-HGG binding by both the thymus and spleen cells was inhibited equivalently with *m*-NBDF and *p*-NBDF. The *p*-NBDF at 5×10^{-5} M concentration was somewhat toxic to the

TABLE V
Inhibitory Effect of DNP Haptens on [¹²⁵I]DNP-HGG Binding by Normal CBA/J Lymphoid Cells

Inhibitor	Concentration	Percent inhibition	
		Spleen	Thymus
	<i>M</i>		
DNP-Lys*	5×10^{-3}	$84 \pm 2\ddagger$	80 ± 6
DNP-Lys	5×10^{-3}	57 ± 3	7 ± 19
<i>m</i> -NBDF§	5×10^{-5}	81 ± 5	77 ± 5
	5×10^{-6}	64 ± 10	43 ± 12
<i>p</i> -NBDF	5×10^{-5}	87 ± 1	—
	5×10^{-6}	77 ± 8	86 ± 7

* Lymphoid cells were not washed after preincubation for 2 h at 4°C.

‡ Standard deviation.

§ DNP affinity labeling compound, meta-nitrobenzenediazonium fluoroborate.

|| DNP affinity labeling compound, para-nitrobenzenediazonium fluoroborate.

T cells. At a concentration of 5×10^{-6} M, the *p*-NBDF was slightly more inhibitory on the thymus and spleen cell binding than was the *m*-NBDF. DNP-Lys, *m*-NBDF, and *p*-NBDF had no significant inhibitory effects on the binding of HGG by spleen cells from HGG-immunized mice. This indicates that these compounds specifically inhibit DNP receptors.

Inhibition of DNP-HGG Binding by Antisera to Immunoglobulin Chains and Fragments.—As previously indicated, the antigen receptor is believed to be immunoglobulin-like. In order to investigate this possibility in relation to the DNP receptors, antisera specific to immunoglobulin chains and fragments were used to inhibit the binding of DNP-HGG by normal spleen and thymus cells. As seen for the spleen cells in Table VI, the anti- γ -chain serum inhibited the antigen binding by 81%. As a control for specificity, the anti- γ -chain serum was absorbed with normal mouse IgG which reduced the inhibition to 12%, a figure similar to that found with normal rabbit serum (21%) and normal goat serum

(15%). Of the other sera specific to immunoglobulin heavy chains, only the anti- μ -chain serum was inhibitory (55%); the anti- α -chain serum had no significant inhibitory effect (15%). The anti-Fab and κ -chain sera were also inhibitory. The inhibitory effect of the anti-Fab serum (84%) was similar to that of the anti- γ -chain serum; the anti- κ serum inhibited by 71%. It is interesting to note that both the anti- γ - and μ -chain sera were inhibitory on the spleen cell binding of DNP-HGG. The effects of the antisera on the binding of DNP-HGG by the normal thymus cells were quite different, as shown in Table VII. The anti- μ -chain sera was inhibitory to 74%; the anti- κ and anti-Fab were also significantly inhibitory, 50 and 63% respectively, but the anti- γ -chain sera had no detectable inhibitory effect.

DISCUSSION

As shown by the above data, significant percentages of cells that specifically bound DNP were detected in the thymuses from normal CBA/J mice. Approxi-

TABLE VI
Inhibitory Effect of Various Antisera on [¹²⁵I]DNP-HGG Binding by Normal CBA/J Mouse Spleen Cells

Antiserum pretreatment*	Percent inhibition
None	0
Normal rabbit serum	21 ± 9‡
Normal goat serum	15 ± 3
Rabbit anti- γ -chain§	81 ± 4
Rabbit anti- γ -chain, abs. γ -chain	12 ± 10
Goat anti-Fab (IgG)	84 ± 6
Goat anti- κ	71 ± 6
Rabbit anti- α -chain	15 ± 10
Rabbit anti- μ -chain	55 ± 23

* 2 h preincubation at 4°C.

‡ Standard deviation.

§ Antisera were heat inactivated and absorbed with CBA/J erythrocytes.

TABLE VII
Inhibitory Effect of Various Antisera on [¹²⁵I]DNP-HGG Binding by Normal CBA/J Mouse Thymus Cells

Antiserum pretreatment*	Percent inhibition
None	0
Rabbit anti- γ -chain‡	-5 ± 29§
Rabbit anti- μ -chain	74 ± 5
Goat anti- κ	50 ± 25
Goat anti-Fab (IgG)	63 ± 20

* 2 h preincubation at 4°C.

‡ Antisera were heat inactivated and absorbed with CBA/J erythrocytes.

§ Standard deviation.

mately 1% of a normal spleen cell preparation and 0.15% of a normal thymus cell preparation bound DNP-HGG. The binding of DNP-HGG by the thymus cells varied from animal to animal, but in all, significant percentages of DNP-HGG-binding cells were detectable. The percentages of antigen-binding cells are higher than most of those previously reported by other investigators for other antigens, especially the percentages obtained with the thymus cell preparations (1-3, 13). Several recent reports of similar high numbers of antigen-binding cells in thymus cell preparations have been reviewed by Miller et al. (12). The question remains, however, whether or not the thymic antigen-binding cells which were detected are truly T cells. In the present study, if all of the DNP-HGG-binding cells in the thymus were immigrant B cells, one would have to assume a 15% contamination of the T cells with B cells assuming that all binding cells in the spleen are B cells. This calculation is based on the percentages of spleen and thymus cells which were shown to bind DNP-HGG. Studies by Roelants (34) indicated that as high as 27% of the normal spleen cells that bound *Maia sequinado* hemocyanin were θ -positive. If this is also true to some extent for DNP-HGG-binding cells in the spleen, then the T cell preparation would have to contain an even greater number of immigrant B cells. This is unlikely, especially since the number of B cells in the thymuses of CBA/J mice according to Unanue³ is less than 0.3%. Alternatively, with such a low percentage of B cells in CBA/J thymuses, there would have to be approximately a 50 times greater proportion of specific DNP-HGG-binding cells in the thymus than is observed in the spleen. This is possible, but seems improbable. Further evidence that the antigen-binding cells in the thymus are T cells was obtained by analysis of the anti- θ -treated thymus cell preparations. If all binders are B cells, one would expect an increase after the killing of T cells. Theoretically, the percentage of antigen-binding B cells should have increased 2-, 5-, and 3-fold for the three anti- θ experiments, respectively (Table II); however, there was no increase in the number of DNP-HGG-binding cells. A 2-fold reduction of antigen-binding T cells was found after killing 80% of T cells, which suggests that the antigen-binding T cells vary in their sensitivities to anti- θ serum and agrees with the work of Raff and Cantor (35) who stated that there are subpopulations of T cells differing in their sensitivities to anti- θ serum.

The higher percentage of cells binding DNP-HGG than other antigens may have been detected because of a higher avidity of this conjugate to the lymphoid cells. The reason for this could be that one DNP-HGG molecule can bind to more than one receptor per cell because of the size of the DNP-HGG conjugate, the number of DNP residues, and other possible specific characteristics of DNP-HGG. The differences in binding avidities and the specific ability of DNP-HGG to bind particularly well are substantiated by the fact that many more spleen and thymus cells bound DNP₅₇-HGG than DNP₁₄-OA. Differences

³ Unanue, E. R. Personal communication.

in binding avidity of various DNP conjugates have also been demonstrated by Davie and Paul (36). These authors have shown that carrier molecules with more DNP residues have a greater avidity for the antigen-binding cells and that the size of the carrier molecule also influences binding avidity. The high numbers of DNP-HGG-binding cells probably also reflect the fact that DNP is a highly immunogenic, haptenic determinant as has been observed by other investigators (21, 22).

The T cells which bound the DNP-HGG were equally proportioned between the T cell subpopulations of cortisone-sensitive and cortisone-resistant cells. The cortisone treatment consistently reduced the T cell population by approximately 95%; the percentage of antigen-binding cells in the remaining thymus did not significantly change. After this treatment there was, however, a small number of cells which bound an unusually large amount of DNP-HGG (> 15 grains/cell). Since the maximum number of such cells that were ever observed in the thymus preparations was $1/10^4$ cells, these particular cells do not seem to be normal constituents of the thymus. They may be B cells which are more easily detected after the extensive reduction of the T cells, or they may be B cells which have immigrated into the thymus after the cortisone treatment since the majority of the positive binding cells in the spleen have more than 15 grains/cell. The failure to find a significant difference in the antigen-binding capabilities of the cortisone-sensitive (cortical T cells) and cortisone-resistant thymus cells (medullary T cells) suggests that the maturation process of the immunocompetent T cell is initiated in the cortex. The theory that T cells gain their immunological competence within the medullary part of the thymus (37, 38) is based on reports (31, 39) which measure T cell functions other than antigen binding. Our results which do not indicate any significant increase in the number of antigen-binding T cells after the cortisone treatment do conflict with the results of Bach and Dardenne (10) and Miller et al. (12) who have shown a significant increase of binding cells after the treatment. It should be noted, however, that different techniques were employed, and therefore the sensitivities of the assays and the populations of cells being detected may have differed.

The binding of DNP-HGG by the thymus and spleen cells was shown to be specific to DNP by inhibition studies. The DNP conjugates had an inhibitory effect, whereas the HGG alone could not inhibit the DNP-HGG binding. The hapten DNP-Lys and the affinity-labeling reagents *m*-NBDF and *p*-NBDF also specifically inhibited the binding of DNP-HGG. If the affinity-labeling compounds *m*-NBDF and *p*-NBDF, which are shown here to bind specifically to DNP receptors, presumably bind covalently to the antigen-combining site of the receptors as they do with the anti-DNP immunoglobulins (32, 33), they may serve as useful tools in future studies for the isolation of the DNP receptor.

The DNP-HGG antigen-binding cells of the thymus appeared to have a lower avidity than the antigen-binding spleen cells. This apparent low avidity of the T cells to antigen could account for the past inability of investigators to

readily detect T cells binding other antigens. Recently, Haskill et al. (40) reported that T cells have low antigen-binding properties and that the bound antigen easily dissociates from the cellular receptors if a fixative is not used; this agrees with our data which indicate that T cells have low avidity for antigens. The low avidity of the T cell could be due to fewer receptors per cell compared with the number of receptors on the B cells and/or lower intrinsic affinity of the individual receptors. It should be emphasized that this comparison is in terms of average avidities (41); possibly some T cells have an avidity for DNP-HGG equal to or greater than some of the cells in the spleen.

The DNP receptors on a large proportion of the spleen cells from CBA/J mice have γ -heavy chains, and the DNP receptors on the thymus cells were mainly μ -chains. No significant differences between the thymic and splenic receptors were detected with anti- κ or anti-Fab sera. Since the DNP receptors on the thymus cells were shown to be mainly μ -chain immunoglobulins and the receptors seemed to have a low avidity for antigen, the cells in the thymus may be in a primary and immunologically undeveloped state. The spleen cells mainly have γ -chain receptors to DNP possibly because of previous DNP stimulation even in the "normal" mice and thus are in a secondary response. The 55% of the spleen cell population which is inhibited by the anti- μ -chain serum may represent T cells in the spleen. The presence of μ -chains on thymus cells has also been reported by other investigators. The surface immunoglobulins isolated from murine thymus cells have been IgM immunoglobulins (42), and Dwyer et al. (13) have shown that the binding of antigen by fetal thymic cells of mice and man is inhibited by anti- μ -chain serum. Different findings on the predominant class of immunoglobulins present on all of the immunoglobulin-positive lymphocytes have been reported; some investigators indicate that the majority of these cells in mice have γ -chain surface immunoglobulins (14), others indicate μ -chains (43), and still others have reported that all immunoglobulin classes are present on the surface of each mouse lymphocyte (44). In the present investigation, the majority of DNP antigen-binding spleen cells appear to have γ -chain receptors. Davie and Paul (3) have shown that in normal guinea pigs the majority of the DNP receptors are also IgG2 immunoglobulins. The anti-immunoglobulin studies are an indirect way to investigate the nature of cell receptors; positive identification of the receptors on T and B cells will rest on their isolation and complete characterization.

SUMMARY

A higher percentage of specific antigen-binding cells can be detected not only in normal CBA/J mouse spleen cell preparations (0.99%), but also in the normal thymus cell preparations (0.15%) with the use of [125 I]2,4-dinitrophenyl-human IgG (DNP-HGG) as compared with most other antigens employed under similar conditions. The receptors on these cells are mainly specific for the DNP group as shown by the inhibition studies with DNP-lysine and the

other DNP conjugates. In addition, it was shown by the inhibition studies with DNP-lysine that the thymus cells seem to have a lower avidity for DNP than the spleen cells. Preincubation of cell suspensions with antisera to immunoglobulins showed that the DNP-HGG antigen-binding cells in the thymus are inhibited predominantly with anti- μ -chain serum and the spleen cells with both anti- μ -chain and anti- γ -chain sera; both cell populations were also significantly inhibited with the antisera to κ -chains and Fab fragments. These data indicate that the nature of the receptor on the T cell differs from that on the majority of spleen cells.

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