STUDIES ON ANTIBODY AFFINITY AT THE CELLULAR LEVEL
CORRELATION BETWEEN BINDING PROPERTIES OF SECRETED ANTIBODY AND
CELLULAR RECEPTOR FOR ANTIGEN ON IMMUNOLOGICAL MEMORY CELLS*

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Similarities between cell-associated antigen-specific receptor and the antibody released by the cell after stimulation have been postulated (1, 2) and such a similarity will, among other things, offer an explanation of the effect of variations in antigen concentration on the affinity of antibodies. If a certain critical concentration of antigen on the cell surface is needed to trigger a precursor cell to high-rate antibody formation, then the antigen concentration will exert a selective pressure during the immune response.

The following indirect evidence for such a cellular selection, mainly obtained from studies on intact animals, is available: (a) antibody affinity increases with time after immunization (3); (b) the immunizing dose regulates the antibody affinity early in the immune response (3, 4); (c) high affinity antibody formation is relatively resistant to suppression by administered antibody (4); (d) high affinity antibody formation is more susceptible to immunological paralysis than low affinity antibody formation (5).

The following indirect evidences for the above outlined postulate obtained from cellular in vitro studies are available: (a) there is a correlation between the affinity of serum antibody of immune cell donor animals and the concentration of antigen needed to trigger the cells into mitosis in vitro (6); (b) an increase with time in immune-cell avidity for antigen has been observed in vitro (7).

However, so far no functional test has been performed showing directly that a cell with a high affinity receptor is also determined to produce a high affinity antibody. The present paper contributes with direct evidence for a parallelism between affinity of cell-associated receptors and antibody released by the same cell after stimulation. Affinity of membrane-located antigen-specific receptors was analyzed by cell fractionation on plastic bead immunoadsorbent columns coated with hapten-protein conjugates (8, 9). Affinity of antibody was determined after transfer to sublethally irradiated host animals and stimulation with antigen. The antibody affinity was studied at the cellular level, using the sensitivity of hemolytic plaque-forming cells to hapten inhibition as a measure of affinity (10, 11).

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Materials and Methods

Animals.—Adult inbred mice of (C3H $\times$ C57BL)F₁ genotype were used. In each experiment mice of the same sex and approximately the same age were used.

Antigens and Immunization.—Bovine serum albumin (BSA)¹ (Cohn fraction V, Koch-Light Laboratories Ltd., Colnbrook, Buckinghamshire, England) and ovalbumin (OA) (Worthington Biochemical Corp., Freehold, N. J., 3 $\times$ crystallized) were used as antigens in their native form or labeled with 4-hydroxy-3-iodo-5-nitrophenylacetic acid (NIP) according to Brownstone et al. (12) or labeled with 2,4-dinitrophenyl (DNP) according to Little and Eisen (13). The procedures for preparation of conjugates of different extents of substitution were as described by these workers. NIP₁₀OA and DNP₁₀OA were used for immunization. For priming of animals, 100 μ g of antigen was injected into each hind footpad in Freund's complete adjuvant (Difco Laboratories, Inc., Detroit, Mich.). Challenge after cell transfer was with 10 μ g of the antigen dissolved in a balanced salt solution (BSS) given mixed with the injected cells.

Preparation of Cell Suspensions.—Adult immune mice were used as cell donors. Spleen and lymph nodes were gently teased and cut so that their content of cells was collected in BSS with 5% rabbit normal serum (RNS). After decantation and washing by centrifugation, the cells were resuspended in BSS with 5% RNS. The cell suspensions were then kept at +4°C until tested or injected.

Transfer of Immunological Memory Cells.—Cell donor animals were immunized as described above and 2–3 months later lymphoid cells were harvested. Recipients were mice that, 1–2 hr before, had been given 600 R whole body irradiation with the following physical factors: 200 kv, 15 ma, focal distance 60 cm, 1.5 mm Al inherent filter, added filter 0.5 mm Cu, half-value layer (HVL) 0.95 mm Cu. The dose rate was 65 R/min. Thereafter lymphoid cells from primed animals were injected intravenously together with antigen dissolved in the buffer. Cell doses and antigen doses were as described for the different experiments in Results.

Column Separation of Lymphoid Cells.—Polymetacrylic plastic bead particles (Degalan) (Wolfgang Degussa Gesellschaft, Hanau, West Germany) were labeled with DNP₄₀BSA by the following procedure. After washing in ordinary tap water the beads were suspended in phosphate-buffered saline (PBS), pH 7.4. The antigen was then added at a concentration of 10 mg/ml. After incubation at 45°C for 1 hr the beads were placed in a refrigerator at +4°C for 18 hr. To the column material, which was approximately 150 ml of plastic beads, was then added 6–10 ml of fetal calf serum. After 1 hr at room temperature the beads were packed in a glass column 1.5 $\times$ 90 cm and free antigen and protein were washed off with PBS. The column was eventually filled with Eagle's medium in Earle's salt solution (EME) and placed at +4°C. A lymphoid cell suspension in 6–10 ml of EME was placed on top of the column and allowed to pass through at a flow rate of 1–2 ml/min. The column separation procedure was essentially the same as that described in the original paper (8).

Assay for Single Antibody-Forming Cells (PFC's).—The hemolytic plaque assay (14) was modified to detect cells producing hapten-specific antibody plaques. Sheep erythrocytes (SRBC) were labeled with NIP-hapten by incubation with 0.5 mg/ml of NIP-azide prepared according to Brownstone et al. (12) in a 0.12 M bicarbonate buffer for 45 min at room temperature. The NIP-SRBC were thereafter washed three times by centrifugation in PBS and finally a 15% suspension in PBS was prepared. Target cells in the DNP-specific plaque assay were

¹ *Abbreviations used in this paper:* AFCP, antibody-forming cell precursor; ARC, antigen-reactive cell; BSA, bovine serum albumin; BSS, balanced salt solution; DNP, 2,4-dinitrophenyl; EACA, ϵ -aminocaproic acid; EME, Eagle's medium in Earle's; I_{50} , hapten concentration inhibiting 50% of PFC's; NIP, 4-hydroxy-3-iodo-5-phenylacetic acid; OA, ovalbumin; PBS, phosphate-buffered saline; PFC, antibody plaque-forming cell; RNS, rabbit normal serum; SRBC, sheep erythrocytes.

SRBC labeled with DNP₄₀BSA prepared according to Little and Eisen (13). The SRBC were incubated with 10 mg/ml of DNP₄₀BSA and 0.275 mg/ml of bisdiazotized benzidine in PBS for 20 min at room temperature. After washing by centrifugation three times in PBS, a 15% suspension was made in PBS containing 10 mg/ml of free BSA. The plaque assay was carried out according to standard procedures (14) using 1.9 ml total volume of 0.7% top-layer agar (Difco Bacto-Agar, Difco Laboratories, Inc., Detroit, Mich.) in EME. 1 mg of diethylaminoethyl (DEAE)-dextran was added per plate. Target erythrocytes were 0.1 ml of a 15% suspension. In the DNP₄₀BSA-SRBC test 1 mg of free BSA was added to the agar together with the erythrocytes. This is a concentration of BSA that will completely inhibit BSA-specific plaques (11). Antibody-forming cell suspension was added in 0.1 ml of EME. After 1 hr at 37°C 3 ml of a rabbit-anti-mouse immunoglobulin serum diluted 1:50 in BSS was added to each plate. After another 15 min at room temperature, 2 ml of fresh guinea pig serum diluted 1:15 in BSS was added to each plate. After 30 min at 37°C the plaques were counted.

During the 1st 2 wk of the primary anti-NIP response, direct plaques were obtained. Since

TABLE I
Variation in the Affinity of the Antibody Response of Mice Immunized with 50 µg of DNP₁₀OA in Each Hind Footpad: Effect at the Cellular Level of Time after Immunization

Day	No. of mice	DNP-PFC's per animal*	Agar plates giving 50% inhibition of PFC's†
		<i>Log₁₀ ± SE</i>	<i>mole/liter</i>
7	3	3.62 ± 0.10	2.06 × 10 ⁻⁵
7	4	3.88 ± 0.31	9.06 × 10 ⁻⁵
12	3	4.09 ± 0.17	2.03 × 10 ⁻⁶
15	4	4.07 ± 0.04	3.51 × 10 ⁻⁶
20	3	4.09 ± 0.05	7.36 × 10 ⁻⁸
37	4	3.62 ± 0.09	3.07 × 10 ⁻⁷
43	3	3.71 ± 0.38	6.08 × 10 ⁻⁸

* Right and left popliteal lymph nodes were pooled. The cells from each animal were tested separately.

† Geometric mean value from separate inhibition tests performed with each individual cell suspension.

we only wanted to study indirect plaques, the direct plaques were inhibited by addition of 0.16 mg concanavalin-A to each plate (15). Later in the anti-NIP response the number of direct plaques was less than 1%. In the anti-DNP system no direct plaques were obtained. The number of direct plaques was negligible in the experiments where secondary responses to NIP or DNP were studied after transfer to irradiated hosts.

Detection of Affinity at the Cellular Level.—The sensitivity of the hemolytic plaques to inhibition with free hapten was used as a measure for affinity (10, 11). Immediately before plating, 0.1 ml of PBS containing 0.001–100 µg of NIP-ε-aminocaproic acid (NIP-EACA) or DNP-ε-aminocaproic acid (DNP-EACA) was added to the top layer of agar. Plates to which no inhibitor had been added served as controls, and the per cent inhibition obtained at the various hapten concentrations was used to calculate the hapten concentration giving 50% inhibition (*I*₅₀).

RESULTS

Demonstration of Heterogeneity of Affinity in the Anti-DNP and Anti-NIP Response at the PFC Level.—As a basis for further experiments we first investi-

gated whether differences in affinity could be demonstrated at the cellular level of mice immunized with DNP₁₀OA or NIP₁₀OA. In Table I it can be seen that an increase in affinity with time after immunization takes place in the anti-DNP system. On day 7 the PFC's showed an I_{50} of 2.06×10^{-5} M free hapten and a gradual increase with time took place. After day 20, when the I_{50} was 7.36×10^{-8} M free hapten, no further increase in affinity was observed. In Table II it can be seen that both antigen dose and time after immunization regulate the affinity in the anti-NIP system. At day 7 after immunization the NIP-PFC's of animals immunized with 5 μ g of NIP₁₀OA had an I_{50} of 5.31×10^{-5} M free hapten, whereas the PFC's of animals immunized with 500 μ g of NIP₁₀OA had an I_{50} of 3.52×10^{-4} M free hapten. Between days 14 and 25 after immunization a gradual increase in affinity was observed

TABLE II
Variation in Affinity of the Antibody Response of Mice Immunized with NIP₁₀OA: Effect at the Cellular Level of Antigen Dose and Time after Immunization

Days after immunization	Antigen dose per footpad	No. of mice	NIP PFC's per animal*	Agar plates giving 50% inhibition of PFC's†
	μ g		$\text{Log}_{10} \pm \text{SE}$	mole/liter
7	5	5	4.16 ± 0.05	5.31×10^{-5}
	500	5	4.32 ± 0.03	3.52×10^{-4}
14	5	4	4.45 ± 0.11	3.74×10^{-6}
	500	4	4.59 ± 0.02	2.11×10^{-5}
25	5	3	4.04 ± 0.21	3.50×10^{-6}
	500	3	4.16 ± 0.04	3.96×10^{-6}

* Right and left popliteal lymph nodes were pooled. The cells from each animal were tested separately.

† Geometric mean value from separate inhibition tests performed with each individual cell suspension.

in both groups and they reached similar values of 3.50×10^{-6} and 3.96×10^{-6} , respectively, at day 25.

In conclusion, the experiments in this section show that a heterogeneity of antibody-binding affinity can be demonstrated at the cellular level in the present anti-DNP and anti-NIP systems of mice.

Fractionation of Immunological Memory Cells of Different Affinity on Hapten-Protein-Coated Columns.—

The reason for addition of excess anti-carrier cells: The humoral antibody response against DNP and NIP in mice is dependent upon an interaction between hapten-specific antibody-forming cell precursors (AFCP's) and carrier protein-specific antigen-reactive cells (ARC's) (16-18). In Table III such a phenomenon is demonstrated in our present single cell system. The purpose of the experiments to be described was to fractionate hapten-specific AFCP's on hapten-coated immunoadsorbent columns. In order to ascertain that the effects observed were due to the fractionation of hapten-specific AFCP's and

not, for example, due to variations in the number of ARC's, an excess number of anti-carrier cells were added to all cell suspensions before injection, together with antigen, into sublethally irradiated animals, i.e. the animals were injected with hapten coupled to the carrier, against which an excess of immune cells had been added.

Affinity fractionation of DNP-specific immunological memory cells on BSA- or DNP-BSA-coated columns: Spleen and lymph node cells from mice primed with DNP-OA 3-4 months earlier were pooled and allowed to pass through columns coated with DNP-BSA and control columns coated with BSA. In Table IV is listed the affinity of antibody released by the cells after transfer to sublethally irradiated recipient mice together with 10^7 anti-OA cells and 10

TABLE III
Hapten-Specific Immunological Memory of Immune Cells Transferred to Sublethally Irradiated Syngeneic Mice: the Effect of Cells Immune to the Protein Carrier on Which the Hapten is Presented

Hapten-specific cells*	Carrier-specific cells*	Immunogen†	NIP-PFC§	DNP-PFC§
			$\text{Log}_{10} \pm \text{SE}$	$\text{Log}_{10} \pm \text{SE}$
Exp. 1				
Anti NIP-OA + anti DNP-OA	10^6	—	$10 \mu\text{g}$ NIP-BSA	1.44 ± 0.14
			+ $10 \mu\text{g}$ DNP-BSA	1.63 ± 0.16
Anti NIPO-A + anti DNP-OA	10^6	Anti BSA 2×10^7	$10 \mu\text{g}$ NIP-BSA	2.73 ± 0.13
			+ $10 \mu\text{g}$ DNP-BSA	2.09 ± 0.20
Exp. 2				
Anti NIP-OA	10^6	—	$10 \mu\text{g}$ NIP-BSA	1.53 ± 0.23
+ anti DNP-OA			+ $10 \mu\text{g}$ DNP-BSA	1.56 ± 0.14
Anti NIP-OA	10^6	Anti BSA 2×10^7	$10 \mu\text{g}$ NIP-BSA	2.66 ± 0.10
+ anti DNP-OA			+ $10 \mu\text{g}$ DNP-BSA	2.97 ± 0.11

* Lymph node and spleen cells from mice immunized with $100 \mu\text{g}$ of antigen in Freund's complete adjuvant 2-3 months earlier. The animals were immunized with one antigen only and mixing was performed in vitro.

† Antigen given in soluble form mixed with the injected cells.

§ PFC's per spleen of the recipients tested 7 days after 600 R and infusion of cells and antigen. Each value is the mean from six animals.

μg of DNP-OA. Unpassed cells and cells passed through the BSA-coated columns released antibody with similar affinity, I_{50} being 6.3×10^{-7} and 2.2×10^{-7} and 2.2×10^{-7} M free hapten, respectively. The cells passed through the DNP-BSA-coated column showed a reduced affinity, with an I_{50} of 2.4×10^{-6} M free hapten. Furthermore, the number of PFC's was also reduced in the DNP-BSA-passed cells, indicating that a hapten-specific elimination of immunological memory cells had taken place, preferentially affecting the precursors of high affinity PFC's.

Affinity fractionation of DNP-specific and NIP-specific immunological memory cells on DNP-BSA-coated columns: The specificity of the elimination on DNP-BSA-coated columns was studied by allowing passage of a mixture of DNP-OA-primed cells and NIP-OA-primed cells. The affinity of antibodies produced after transfer to sublethally irradiated recipients was thereafter

studied against both the DNP and the NIP specificities. Further, possible effects on the affinity caused by variations in the number of hapten-specific memory cells were controlled by transferring different doses of unpassed and passed cells. As in previous experiments an excess of anti-OA cells was added to

TABLE IV
Affinity Fractionation of DNP-Specific Immunological Memory Cells on BSA or DNP-BSA-Coated Columns

Cells*	Log ₁₀ DNP-PFC $\pm$ SE†	Hapten giving 50% inhibition§ <i>mole/liter</i>
Control	3.21 $\pm$ 0.27	6.31 $\times 10^{-7}$
Passed BSA column	3.49 $\pm$ 0.12	2.18 $\times 10^{-7}$
Passed BSA-DNP column	2.70 $\pm$ 0.08	2.40 $\times 10^{-6}$

* Pooled spleen and lymph node cells from mice immunized with DNP-OA 3-4 months earlier. Each animal received 10^6 such cells and 2×10^7 cells from animals immunized with OA 3-4 months earlier. Each animal received 10 μ g of dissolved DNP-OA mixed with the cells.

† Number of PFC's per spleen 7 days after 600 R and infusion of cells and antigen. Each value is the mean of four to five different mice.

§ Geometric mean from inhibition tests performed with cells from individual animals.

TABLE V
Affinity Fractionation of DNP-Specific and NIP-Specific Immunological Memory Cells on DNP-BSA-Coated Columns

Cell transfer		DNP response		NIP response		DNP-PFC/ NIP-PFC	
Cells*	Cell dose	Log ₁₀ PFC† $\pm$ SE	Hapten giving 50% inhibition§ <i>mole/liter</i>	Log ₁₀ PFC† $\pm$ SE	Hapten giving 50% inhibition§ <i>mole/liter</i>	Ratio	Reduction %
Control	10^7	3.72 $\pm$ 0.08	2.91 $\times 10^{-7}$	4.61 $\pm$ 0.05	8.71 $\times 10^{-6}$	0.13	—
	5×10^6	3.59 $\pm$ 0.04	2.46 $\times 10^{-6}$	4.25 $\pm$ 0.11	9.80 $\times 10^{-6}$	0.23	—
	2.5×10^6	3.21 $\pm$ 0.28	3.52 $\times 10^{-6}$	4.01 $\pm$ 0.15	9.59 $\times 10^{-6}$	0.17	—
Passed DNP-BSA column	10^7	3.21 $\pm$ 0.10	2.75 $\times 10^{-6}$	4.37 $\pm$ 0.04	2.55 $\times 10^{-6}$	0.07	44
	5×10^6	3.10 $\pm$ 0.11	3.16 $\times 10^{-6}$	3.97 $\pm$ 0.10	3.56 $\times 10^{-6}$	0.15	38
	2.5×10^6	2.80 $\pm$ 0.08	1.51 $\times 10^{-4}$	3.67 $\pm$ 0.07	7.89 $\times 10^{-6}$	0.14	20

* Pooled spleen and lymph node cells from mice immunized with DNP-OA and NIP-OA 3-4 months earlier. Each animal received hapten primed cell dose as indicated and in addition 2×10^7 cells from animals immunized with OA 3-4 months earlier. Dissolved antigen was given mixed with the cells, 10 μ g of DNP-OA and 10 μ g of NIP-OA.

† Number of PFC's per spleen 7 days after 600 R and infusion of cells and antigen. Each value is the mean of four to five different mice.

§ Geometric mean from inhibition tests performed with cells from individual animals.

the transferred cell suspensions and stimulation was with 10 μ g of soluble DNP-OA and 10 μ g of NIP-OA. In Tables V, VI, and VII such experiments are shown. A specific reduction of anti-DNP memory cells was observed. Further, a preferential elimination of high affinity PFC precursors was observed. The effect of this was that the average affinity of passed anti-DNP PFC's was

TABLE VI
*Affinity Fractionation of DNP-Specific and NIP-Specific Immunological
 Memory Cells on DNP-BSA-Coated Columns*

Cell transfer		DNP response		NIP response		DNP-PFC/ NIP-PFC	
Cells*	Cell dose	Log ₁₀ PFC† ± SE	Hapten giving 50% inhibition‡ <i>mole/liter</i>	Log ₁₀ PFC† ± SE	Hapten giving 50% inhibition‡ <i>mole/liter</i>	Ratio	Reduction %
Control	10 ⁷	3.50 ± 0.14	3.33 × 10 ⁻⁷	4.23 ± 0.12	8.98 × 10 ⁻⁶	0.19	—
	5 × 10 ⁶	2.94 ± 0.13	1.18 × 10 ⁻⁶	3.82 ± 0.10	2.43 × 10 ⁻⁵	0.13	—
	2.5 × 10 ⁶	2.55 ± 0.10	6.19 × 10 ⁻⁵	3.47 ± 0.10	3.03 × 10 ⁻⁵	0.12	—
Passed DNP- BSA column	10 ⁷	3.11 ± 0.10	2.83 × 10 ⁻⁵	4.34 ± 0.10	1.29 × 10 ⁻⁵	0.06	68
	5 × 10 ⁶	3.04 ± 0.09	5.16 × 10 ⁻⁴	4.31 ± 0.12	1.43 × 10 ⁻⁵	0.06	59
	2.5 × 10 ⁶	2.32 ± 0.14	1.36 × 10 ⁻⁴	3.74 ± 0.08	3.50 × 10 ⁻⁵	0.04	68

* Pooled spleen and lymph node cells from mice immunized with DNP-OA and NIP-OA 3-4 months earlier. Each animal received hapten primed cell dose as indicated and in addition 2 × 10⁻⁷ cells from animals immunized with OA 3-4 months earlier. Dissolved antigen was given mixed with the cells, 10 μg of DNP-OA and 10 μg of NIP-OA.

† Number of PFC's per spleen 7 days after 600 R and infusion of cells and antigen. Each value is the mean of four to five different mice.

‡ Geometric mean from inhibition tests performed with cells from individual animals.

TABLE VII
*Affinity Fractionation of DNP-Specific and NIP-Specific Immunological
 Memory Cells on DNP-BSA-Coated Columns*

Cell transfer		DNP response		NIP response		DNP-PFC/ NIP-PFC	
Cells*	Cell dose	Log ₁₀ PFC† ± SE	Hapten giving 50% inhibition‡ <i>mole/liter</i>	Log ₁₀ PFC† ± SE	Hapten giving 50% inhibition‡ <i>mole/liter</i>	Ratio	Depres- sion %
Control	10 ⁷	4.10 ± 0.10	1.68 × 10 ⁻⁸	4.55 ± 0.10	1.14 × 10 ⁻⁵	0.35	—
	3 × 10 ⁶	3.05 ± 0.08	3.01 × 10 ⁻⁷	3.34 ± 0.09	2.36 × 10 ⁻⁶	0.52	—
Passed DNP- BAS column	10 ⁷	3.46 ± 0.12	3.37 × 10 ⁻⁶	4.45 ± 0.04	4.20 × 10 ⁻⁶	0.10	71
	3 × 10 ⁶	2.36 ± 0.17	3.83 × 10 ⁻⁶	3.78 ± 0.10	5.03 × 10 ⁻⁶	0.04	92

* Pooled spleen and lymph node cells from mice immunized with DNP-OA and NIP-OA 3-4 months earlier. Each animal received hapten primed cell dose as indicated and in addition 2 × 10⁻⁷ cells from animals immunized with OA 3-4 months earlier. Dissolved antigen was given mixed with the cells, 10 μg of DNP-OA and 10 μg of NIP-OA.

† Number of PFC's per spleen 7 days after 600 R and infusion of cells and antigen. Each value is the mean of four to five different mice.

‡ Geometric mean from inhibition tests performed with cells from individual animals.

lower than control PFC's. The anti-NIP PFC's were not affected quantitatively or qualitatively by passage through the DNP-coated columns.

In Fig. 1 the distribution of high and low affinity antibody-forming cells has been compared in spleens from mice receiving control memory cells and memory cells passed through a hapten-coated column. It can be seen that the main difference between the two cell populations is that high affinity antibody-

forming cells are virtually absent in the group receiving passed cells. This strongly indicates that high affinity memory cells show a higher tendency to stick in the immunoadsorbent columns. The most likely reason for this is that their hapten-specific receptors have a higher affinity for the antigen.

DISCUSSION

The present data illustrate in a direct way that immunological memory cells in the anti-DNP system of mice are specialized with regard to affinity. The specialization is shown to occur at the level of the cell-associated hapten-specific receptor of these cells, since it was possible to show a selective reten-

FIG. 1. Distribution of DNP-PFC's in spleens of mice receiving either control memory cells or memory cells passed through a DNP column. The PFC's are sorted into groups, being sensitive to inhibition in different hapten concentration intervals; e.g., the numbers in the 0.01-0.1 columns represent PFC's resistant to 0.01 μ g but inhibitable by 0.1 μ g.

tion of high affinity memory cells in immunoadsorbent columns coated with hapten-protein conjugate.

Another explanation for the selective retention of high affinity memory cells is a higher density of receptors on the high affinity cells. This, however, seems unlikely in view of quantitative studies on antigen-binding cells where no such increase in receptor density was observed (19).

The present anti-hapten systems were found, in confirmation of previous reports (16-18), to consist of two cooperative types of cells, ARC's, which are immune to the carrier protein, and AFCP's which have hapten specificity. In the affinity fractionation experiments described in this article an excess of anti-carrier reactive cells was always ascertained by addition of such cells. The differences in affinity observed thus seem to exist at the level of the AFCP's, which is in accordance with earlier indirect evidence on this point (20).

Experiments are also in progress to determine whether nonimmune AFCP's as well can be functionally fractionated on immunoadsorbent columns. There is reason to believe that the performance of such a fractionation will be possible, since it has recently been shown that antigen-binding lymphocytes of nonimmune, as well as immune, guinea pigs are heterogeneous with regard to binding affinity of their membrane receptors (21, 22).

In summary the direct evidence for similarities between membrane-located receptor antibody of lymphocytes (AFCP's) and the humoral antibody released by the cell are the following. (a) The receptors have been shown to have the same specificity as the humoral antibody released by the cells (8). (b) The receptors are immunoglobulins and express the same class specialization as the immunoglobulins released by the cells (23). (c) The receptor discriminates a haptenic site on a hapten-protein conjugate to the same extent as does the humoral antibody released by the cell (9). (d) The receptor expresses the affinity for the antigen, and immunological memory cells express similar affinity of their membrane receptor and their humoral antibody released after stimulation (as shown in this article).

SUMMARY

Heterogeneity with regard to affinity of anti-hapten antibody was demonstrated at the cellular level in mice. The heterogeneity was shown at the level of single antibody-forming cells using hapten inhibition of hemolytic antibody plaque formation as a measure of affinity. The affinity increased with time after immunization. A high antigen dose initially resulted in relatively low affinity antibody production as compared to the affinity of the antibody production in animals immunized with a low dose.

Affinity specialization of immunological memory cells was demonstrated, since it was possible to specifically fractionate such cells with regard to affinity on hapten-protein-coated plastic bead columns. High affinity memory cells showed a higher tendency to become retained in the columns than did low affinity memory cells.

The data in a direct way demonstrate that memory cells carrying membrane-associated receptors of a certain affinity for the antigen are determined to release antibody of a similar affinity after stimulation with antigen.

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