

THE ROLE OF THYMOCYTES AND BONE MARROW CELLS IN
DEFINING THE RESPONSE TO THE DINITROPHENYL
HAPTEN ATTACHED TO POSITIVELY AND NEGATIVELY
CHARGED SYNTHETIC POLYPEPTIDE CARRIERS

CELL FRACTIONATION OVER CHARGED COLUMNS

BY YUVAL KARNIELY, EDNA MOZES, G. M. SHEARER,* AND MICHAEL SELA

(From the Department of Chemical Immunology, The Weizmann Institute of Science,
Rehovot, Israel)

(Received for publication 5 September 1972)

An inverse relationship has been demonstrated between the net electrical charge of immunogens and the charge of the antibodies elicited by them (1-3). This phenomenon was shown for antibodies of the IgM (4), IgG (1), and IgE (3) classes, and was found in a number of species, including rabbits (1, 2), mice (5), goats (6), and humans (3). IgG antibodies to natural and synthetic negatively charged immunogens were found in the first, more basic, fraction of immunoglobulin eluted from diethylaminoethyl-Sephadex A-50. In contradistinction, the antibodies elicited by basic immunogens appeared in the second, more acidic, eluted fraction (1-5). This observation has been extended to include antihapten antibody responses generated by groups such as 2,4-dinitrophenyl (DNP)¹ and a peptide of D-alanine attached to positively and negatively charged carriers (1, 2, 7). These hapten-specific antibodies differed in net electrical charge, but were indistinguishable with respect to their specificity and affinity (7), strongly suggesting that, over and above the selection of the antibody-combining site by the antigenic determinant, the "carrier" portion of the immunogen largely determines the chemical nature of the antibody formed (1).

It was of interest to establish whether the inverse net charge phenomenon has a cellular basis. Column chromatography of lymphoid cells bearing antibodylike receptors has been achieved by attaching antigenic molecules to glass, plastic (8-10), polyacrylamide beads (11, 12), and fibers (13). The objective of such studies has been to deplete and/or enrich antigen-sensitive cell populations (9, 10). Should a population of immunocompetent cells exist that can discriminate between immunogenic macromolecules as a function of their charge properties, in addition to or instead of recognition based on antibody specificity, the more positively charged cells would be expected to react preferentially with more acidic immunogens, and vice versa. In a previous study an inverse net charge relationship was demonstrated at the cellular level (14).

* Present address: Immunology Branch, National Cancer Institute, National Institutes of Health, Bethesda, Md. 20014.

¹ Abbreviations used in this paper: DNP, 2,4-dinitrophenyl; DNP-901 (T,G,L), dinitrophenylated poly(Glu⁸³Lys¹²Tyr⁵) acidic synthetic copolymer; DNP-912 (T,G,L), dinitrophenylated poly(Glu⁴⁸Lys⁷⁵Tyr⁶) basic copolymer.

Mouse spleen cells passed over negatively charged glass bead columns exhibited a reduced immune response potential to DNP as compared with unfractionated cells when the hapten was attached to a negatively charged synthetic polypeptide carrier. No reduction, as a result of cell fractionation over glass beads, was detected in anti-DNP response when the hapten was attached to a positively charged synthetic polypeptide (12). Furthermore, the DNP-specific antibodies produced by the fractionated cells after immunization with the DNP-acidic conjugate were predominantly acidic (14).

The objectives of this investigation were to verify the charge-dependent cell fractionation using positively charged columns, and to determine whether the cellular basis of the net electrical charge phenomenon could be attributed to cells of thymus and/or bone marrow origin. Spleen cell fractionation on positively charged poly-L-lysine-coated glass beads resulted in a marked reduction in immune response potential to the basic DNP-carrier conjugate, but not to DNP on an acidic carrier. A reduction in responsiveness was obtained using cell mixtures of unfractionated marrow cells and thymocytes fractionated on glass bead (negatively charged) or poly-L-lysine-coated glass bead (positively charged) columns, only when the recipient mice were immunized with DNP conjugated to a carrier of like charge. However, no reduction in responsiveness to DNP on either carrier was detected when mixtures of fractionated marrow cells and unfractionated thymocytes were injected.

Materials and Methods

Antigens.—The acidic synthetic copolymer DNP-901 (T,G,L) is a dinitrophenylated poly(Glu⁸³Lys¹²Tyr⁵) (all L-amino acid residues; average mol wt 54,000) and contains 4.4 mol of dinitrophenyl per mol of 901 (T,G,L) (1). DNP-912 (T,G,L) is a basic copolymer dinitrophenyl poly(Glu¹⁹Lys⁷⁵Tyr⁶) of average mol wt 65,000, containing 6.0 dinitrophenyl mol (14). These two copolymers were used as the immunogens. DNP-bovine serum albumin (crystallized; Armour Pharmaceutical Co., Chicago, Ill.) containing on the average 7.5 DNP mol/mol of protein was used as the cross-reacting antigen for titration of DNP-specific antibodies.

Preparation of Negatively and Positively Charged Columns.—Columns of uncoated glass beads (Superbrite, 100-5005; Minnesota Mining & Manufacturing Co., St. Paul, Minn.) were prepared according to the method described by Wigzell and Andersson (8). Poly-L-lysine-coated glass beads were prepared by incubation in a solution of 5 mg/ml poly-L-lysine hydrochloride, average degree of polymerization 95, in phosphate-buffered saline (0.15 N NaCl, 0.01 M phosphate buffer, pH 7) for 1 h at 45°C, and then for 18 h at 4°C. After incubation the poly-L-lysine solution was removed, and the coated beads were washed in phosphate-buffered saline until the supernatant contained no detectable poly-L-lysine as measured by absorbance at 230 nm. The columns consisted of glass pipettes, 22.5 cm long × 0.86 cm diameter. Fine wire mesh was placed in the bottom of each pipette to retain the beads. The void volumes of the columns packed with beads was 4 ml.

Cell Preparations and Fractionation on Columns.—Cell suspensions were prepared from the spleen, thymus, and bones of the hindlegs of 8-10-wk old female (BALB/c × C57BL/6)F₁ donor mice as described elsewhere (15, 16). The concentration of each cell suspension was estimated by repeated sample counting of nucleated cells using a hemacytometer. The cells were passed over the columns at 4°C. Each column was loaded with 1×10^8 spleen, 1×10^8 bone marrow, or $1.5-2.0 \times 10^8$ thymus cells, in a total volume of 0.75-1.5 ml. The unbound

cells were eluted with cold Eagle's medium. The flow rate through the column was 3–4 ml/min. The cell suspensions were recounted and adjusted to the desired concentrations after filtration. In the studies involving the passage of thymus and marrow cells over the charged columns, filtered thymocytes were mixed only with unfiltered marrow cells, and filtered marrow cells were mixed only with untreated thymocytes for assessment of immunocompetence in irradiated recipient mice.

Cell Transfers and Immunization.—(BALB/c × C57BL/6) F_1 recipient mice of both sexes, 10–12 wk of age, were exposed to 750–800 rad of ^{60}Co gamma irradiation. The mice were injected via the tail vein with varying numbers of filtered or unfiltered spleen cells, with graded inocula of filtered or unfiltered thymocytes mixed with 2×10^7 unfiltered marrow cells, or with graded inocula of filtered or unfiltered marrow cells mixed with 2×10^8 unpassed thymocytes. The recipients were immunized intraperitoneally with 10 μg of either the acidic DNP-901 (T,G,L,) or the basic DNP-912 (T,G,L,) in complete Freund's adjuvant (Difco Laboratories, Detroit, Mich.). 2 wk after cell transfer (at the time of peak anti-DNP titers), the recipients were bled and their sera individually assayed for DNP-specific antibodies by passive microhemagglutination.

Passive Microhemagglutination Assay.—Sheep erythrocytes were formalinized, tanned, and coated with DNP-bovine serum albumin (17). The hemagglutinations were performed on disposable microtiter plates (Cooke Engineering Co., Alexandria, Va.) by twofold serial dilutions of antisera in phosphate-buffered saline containing 0.1% normal rabbit serum. The plates were incubated at 20°C and read after 2 h. Anti-DNP titers in irradiated control animals injected with cells or antigen only were not detected at dilutions greater than 1:4. Therefore, donor-derived responses showing titers at dilutions greater than 1:4 in recipient sera were considered to be positive.

RESULTS

Effect of Spleen Cell Filtration over Poly-L-Lysine-Coated Glass Beads on Immune Response to DNP.—The results of a previous study have indicated a cellular basis for the net charge correlation between immunogens and the antibodies produced by spleen cells after filtration of the cells over negatively charged glass bead columns (14). It was desirable to verify the above finding in which more basic cells were depleted from the cell population by filtration of spleen cells over positively charged columns, where it was hoped that the more acidic cells would be retained. Glass beads were coated with poly-L-lysine, and the cells (10^8 /column) were passed over columns prepared from these positively charged beads. Approximately 80% of the passed cells were retained by the columns.

After filtration, the cells that passed through the columns, as well as unfiltered spleen cells (controls), were adjusted to the desired concentrations and injected into groups of irradiated, syngeneic hosts. The recipients of the filtered and unfiltered cells were divided into two groups: one group was immunized with DNP-901 (T,G,L) (i.e. DNP attached to a negatively charged carrier) and the other with DNP-912 (T,G,L) (i.e. DNP attached to a positively charged carrier). The recipients were bled 2 wk later. The percentage of sera showing anti-DNP titers at a dilution greater than 1:4 (i.e. above the radiation control levels) are summarized in Table I for the four groups of irradiated mice that received different inocula of spleen cells ($1 - 10 \times 10^8$).

TABLE I

*Percentage of Positive Sera in Irradiated Mice after Transfer of Unfiltered or of Poly-L-Lysine-Coated Glass Bead-Filtered Spleen Cells and Immunization with Dinitrophenylated Acidic or Basic Copolymers**

no. of spleen cells transferred ($\times 10^6$)	Unfiltered cells				Cells filtered over poly-L-lysine-coated glass beads			
	Immunogen							
	DNP-901(T, G, L) (negative)		DNP-912(T, G, L) (positive)		DNP-901(T, G, L) (negative)		DNP-912(T, G, L) (positive)	
	Fraction of positive sera	Percent of positive sera	Fraction of positive sera	Percent of positive sera	Fraction of positive sera	Percent of positive sera	Fraction of positive sera	Percent of positive sera
1	2	3	4	5	6	7	8	9
		%		%		%		%
1	8/15	53 \pm 13	7/14	50 \pm 13	6/13	46 \pm 14	2/14	14 \pm 9
2	7/14	50 \pm 13	8/14	57 \pm 13	5/9	56 \pm 17	3/15	20 \pm 10
5	9/14	64 \pm 13	8/13	62 \pm 13	11/15	73 \pm 11	5/12	41 \pm 14
10	12/15	80 \pm 10	15/19	79 \pm 9	12/17	71 \pm 11	8/18	44 \pm 12

\pm Standard error of ratio.

* Donor-derived responses showing anti-DNP hemagglutination titers at dilutions greater than 1:4 in recipient sera.

As the inoculum of spleen cells was increased there was a general increase in the percentage of recipients that responded to DNP.

No distinction could be made between the anti-DNP response frequencies generated by any inoculum of unfiltered cells as a function of the negatively or positively charged carrier portions of these immunogens. In contrast, after filtration over the positively charged columns, the percentage of anti-DNP responses generated after immunization with the hapten conjugated to the positively charged carrier was significantly lower for all four inocula tested than the percentage of responses generated after immunization with the negatively charged dinitrophenylated copolymer. Cell fractionation had no detectable effect on the response frequency to the latter immunogen when compared with the frequencies generated in recipients injected with unfiltered cells and immunized with either immunogen. These results indicate that spleen cell filtration over a positively charged bead column reduced the response potential to DNP when this hapten was attached to a carrier of positive net electrical charge, but did not affect the responsiveness to the same hapten linked to a carrier of negative charge.

Since the mouse spleen is composed of heterogeneous populations of immunocompetent cells with distinct functions (18, 19) and of different anatomical origin (20, 21), it was of interest to determine whether the net charge correlation could be associated with a population of thymus-derived and/or marrow-derived cells. The anti-DNP responses to DNP-901 (T, G, L) and to DNP-912 (T, G, L) were both found to be thymus dependent, since no antibody responses were detected in irradiated mice injected extensively with 2×10^7

marrow cells and immunized with either immunogen. In contrast, significant anti-DNP titers were detected in more than 90% of the recipients injected with 10^8 thymocytes plus 2×10^7 marrow cells, and immunized with these two antigens.

Effect of Thymus Cell Filtration over Glass Beads or Poly-L-Lysine-Coated Glass Beads on Immune Response to DNP.—Thymocytes (1.5×10^8 /column) were passed over columns of glass beads or of poly-L-lysine-coated glass beads. The percentages of thymus cells not retained by the columns were 46 and 28, respectively. Irradiated recipient mice were injected with 2×10^7 unfiltered marrow cells mixed with graded inocula (1.5 – 6.0×10^7) of unfiltered, glass bead-filtered, or poly-L-lysine glass bead-filtered thymocytes. Each group of recipients was divided into two groups: one was immunized with the acidic DNP-901 (T,G,L) and the other with the basic DNP-912 (T,G,L). The fraction and percentage of anti-DNP responses detected are shown in Table II. The proportion of responses observed increased as the thymocyte inoculum was increased. The anti-DNP response frequencies obtained with unfiltered thymocytes were indistinguishable throughout the inoculum range, irrespective of whether the hapten was administered on the acidic or on the basic carrier. Similar response frequencies were obtained in recipients of unfiltered marrow cells and glass bead-filtered thymocytes immunized with DNP on the basic copolymer. In contrast, a significant reduction in the percentage of anti-DNP responses was detected in recipients of the same cell mixtures immunized with DNP on the acidic copolymer (cf. column 7 with columns 3, 5, and 9 of Table II).

Filtration of thymocytes over poly-L-lysine-coated glass beads had an opposite effect on the response to DNP in recipients immunized with the acidic and basic immunogens. No reduction in responsiveness was observed in irradiated mice injected with mixtures of unfiltered marrow cells and thymocytes passed over the positively charged beads immunized with DNP-901 (T,G,L). However, the recipients of these cell mixtures immunized with the basic DNP-912 (T,G,L) exhibited reduced anti-DNP response frequencies (cf. the last column of Table II with columns 3, 5, 9, and 11). These results demonstrate a reduction in responsiveness to DNP only when the carrier portion of the immunogen and the column over which the cells were filtered are of like charge, and they imply the existence of thymocyte populations which recognize immunogens based on their overall electrical charge.

Effect of Marrow Cell Filtration over Glass Beads or Poly-L-Lysine-Coated Glass Beads on Immune Response to DNP.—Bone marrow cells (10^8 /column) were passed over glass bead or over poly-L-lysine-coated glass bead columns. The cell yields after filtration were respectively 25 and 12%. Recipients were injected with 10^8 unfiltered thymocytes mixed with different numbers (1 – 15×10^6) of: (a) unfiltered marrow cells, (b) marrow cells passed over glass bead columns, or (c) marrow cells passed over poly-L-lysine-coated glass beads.

TABLE II
*Percentage of Positive Sera in Irradiated Mice after Transfer of Unfiltered, Glass Bead-Filtered, or Poly-L-Lysine-Coated Glass Bead-Filtered Thymocytes Mixed with 2×10^7 Unfiltered Marrow Cells and Immunization with Dinitrophenylated Acidic or Basic Copolymers**

no. of thymocytes transferred ($\times 10^6$)	Unfiltered cells			Thymocytes filtered over glass beads			Thymocytes filtered over poly-L-lysine-coated glass beads					
	Immunogen			Immunogen			Immunogen					
	DNP-901(T, G, L)	DNP-912(T, G, L)	DNP-901(T, G, L)	DNP-901(T, G, L)	DNP-912(T, G, L)	DNP-901(T, G, L)	DNP-901(T, G, L)	DNP-912(T, G, L)	DNP-912(T, G, L)			
1	Fraction of positive sera 2	Fraction of positive sera 4	Fraction of positive sera 5	Fraction of positive sera 6	Fraction of positive sera 7	Fraction of positive sera 8	Fraction of positive sera 9	Fraction of positive sera 10	Fraction of positive sera 11	Fraction of positive sera 12	Fraction of positive sera 13	
	Percent of positive sera 3	Percent of positive sera 4	Percent of positive sera 5	Percent of positive sera 6	Percent of positive sera 7	Percent of positive sera 8	Percent of positive sera 9	Percent of positive sera 10	Percent of positive sera 11	Percent of positive sera 12	Percent of positive sera 13	
	%	%	%	%	%	%	%	%	%	%	%	
15	19/44	43 ± 6	17/42	41 ± 8	1/31	3.0 ± 2	12/29	41 ± 9	6/16	38 ± 12	2/15	13 ± 8
30	16/35	46 ± 8	15/34	44 ± 7	1/14	7.0 ± 2	6/15	40 ± 13	9/19	47 ± 11	3/19	16 ± 8
45	23/34	68 ± 8	19/35	54 ± 8	2/11	18 ± 12	9/15	60 ± 13	12/20	60 ± 11	6/20	30 ± 10
60	21/25	78 ± 6	21/26	81 ± 8	12/34	35 ± 8	10/11	91 ± 9	9/13	70 ± 13	5/15	33 ± 12

± Standard error of ratio.

* Donor-derived responses showing anti-DNP hemagglutination titers at dilutions greater than 1:4 in recipient sera.

TABLE III
*Percentage of Positive Sera in Irradiated Mice after Transfer of Unfiltered, Glass Bead-Filtered, or Poly-L-Lysine-Coated Glass Bead-Filtered Marrow Cells Mixed with 10^8 Unfiltered Thymocytes and Immunization with Dinitrophenylated Acidic or Basic Copolymers**

no. of marrow cells transferred ($\times 10^6$)	Unfiltered cells			Marrow cells filtered over glass beads			Marrow cells filtered over poly-L-lysine-coated glass beads					
	Immunogen			Immunogen			Immunogen					
	DNP-901(T, G, L)	DNP-912(T, G, L)	DNP-901(T, G, L)	DNP-901(T, G, L)	DNP-912(T, G, L)	DNP-901(T, G, L)	DNP-901(T, G, L)	DNP-912(T, G, L)	DNP-912(T, G, L)			
1	Fraction of positive sera 2	Fraction of positive sera 3	Fraction of positive sera 4	Fraction of positive sera 5	Fraction of positive sera 6	Fraction of positive sera 7	Fraction of positive sera 8	Fraction of positive sera 9	Fraction of positive sera 10	Fraction of positive sera 11	Fraction of positive sera 12	Fraction of positive sera 13
	Percent of positive sera 3	Percent of positive sera 4	Percent of positive sera 5	Percent of positive sera 6	Percent of positive sera 7	Percent of positive sera 8	Percent of positive sera 9	Percent of positive sera 10	Percent of positive sera 11	Percent of positive sera 12	Percent of positive sera 13	
	%	%	%	%	%	%	%	%	%	%	%	
1	7/18	39 ± 11	6/15	40 ± 13	N.D.	N.D.	N.D.	N.D.	7/20	35 ± 10	6/18	33 ± 11
3	3/11	27 ± 13	N.D.	N.D.	2/8	25 ± 15	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
5	23/47	49 ± 7	25/44	57 ± 7	18/35	52 ± 8	17/34	50 ± 9	7/11	64 ± 14	7/12	58 ± 14
10	8/15	53 ± 13	6/15	40 ± 13	7/15	47 ± 13	8/15	53 ± 13	N.D.	N.D.	N.D.	N.D.
15	8/14	57 ± 13	5/11	45 ± 15	5/9	55 ± 17	7/15	47 ± 13	N.D.	N.D.	N.D.	N.D.

± Standard error of ratio.

N.D. = not done.

* Donor-derived responses showing anti-DNP hemagglutination titers at dilutions greater than 1:4 in recipient sera.

Half of each of the three groups of host mice was immunized with DNP-901 (T,G,L) and the other half with DNP-912 (T,G,L). The response frequencies generated are summarized in Table III. No proportional change in response frequency was detected as a function of the marrow cell inoculum in any of the groups of recipients. Unlike the results obtained using fractionated spleen cells (Table I and ref. 14) or thymocytes (Table II), no reduction in anti-DNP responses was detected in recipients of unfiltered thymocytes mixed with marrow cells filtered over columns of either charge.

Fractionation of bone marrow cells over charged columns had no apparent effect on the response to the hapten attached to carriers of unlike charge. However, the possibility must be considered that marrow-derived cells residing in the spleen could have differentiated to a stage in which they are capable of recognizing the charge properties of immunogens. In order to test this hypothesis, thymectomized, irradiated, recipient mice were injected with 5×10^6 syngeneic bone marrow cells. Cell suspensions were prepared from the chimeric spleens 6 wk later, and samples were passed over the negatively charged columns (10^8 /column). 10^7 filtered or unfiltered marrow-derived spleen cells were mixed with 10^8 unfractionated thymocytes and injected into irradiated test recipients, which were then immunized with DNP-901 (T,G,L) or DNP-912 (T,G,L). No significant differences were observed in the percentage of responses to DNP on either carrier in recipients of the filtered or unfiltered marrow-derived cells. The percentage of responses of the four groups ranged between $60 \pm 11\%$ and $79 \pm 9\%$. Thus, no evidence was found, using these experimental protocols, for the existence of a marrow-derived population of immunocytes which recognizes immunogens based on their net electrical charge.

DISCUSSION

Functionally distinct populations of immunocompetent cells have been identified in the lymphoid tissues of mice and guinea pigs (18, 20-28). The functions that have been characterized until now include the production of specific antibodies by bone marrow-derived precursors of antibody-forming cells (18), and the carrier or helper functions (23-25), as well as the suppressive effects (26) attributed to thymus-derived cells. These reports have involved: (a) microscopic identification of receptors on lymphocyte surfaces (29-31), (b) isolation of immunoglobulin-like material from lymphoid cell membranes (32), (c) inhibition of immunological functions by anti-immunoglobulin (28, 33, 34), and (d) separation of lymphoid cells on antigen-coated columns (8-13, 27). In these latter experiments, the lymphoid cell populations from spleen and bone marrow were fractionated on the basis of antigenic specificity (8, 13). Attempts to separate thymocytes bearing antibodylike receptors, however, have been unsuccessful (10), although reports indicate that thymus cells involved in cellular immune reactions can be fractionated using monolayers of cells of a different *H-2*-type (35-37). Recently, lymphoid subpopulations

expressing receptors for histamine have been isolated from mouse spleen (38) and human peripheral blood (39). Thus, it may be possible to fractionate immunocompetent cells on the basis of surface receptors other than those resembling antibody.

The results reported here of spleen cell fractionation over positively charged poly-L-lysine-coated glass bead columns verify a previous study in which spleen cells were passed over a column of negatively charged glass beads (14). Fractionation of spleen cells over the positively charged column reduced the percentage of anti-DNP responses in irradiated recipients inoculated with filtered cells and immunized with a conjugate of DNP on the positively charged copolymer [DNP-912 (T,G,L)]. However, it did not affect responsiveness to DNP attached to the negative 901 (T,G,L) (see Table I). Since filtered cells are assumed to be more positive, an inverse correlation has been established between the net electrical charge of the immunogen and that of the spleen cells responding to these immunogens after filtration over charged columns.

Cells of thymic origin appear to function as modifiers of antibody responses. Helper cell functions for a number of immunogens, including heterologous erythrocytes (20, 21), proteins (40), synthetic polypeptides composed of L-amino acids (16, 41), and hapten-carrier conjugates (23-25) have been attributed to thymus-derived cells. The net charge phenomenon is associated with properties of the entire antigenic macromolecule, and is not related to the specificity of the antibodies produced (14). Therefore, it was expected that fractionation of thymocytes over charged columns would affect the anti-DNP response in a manner similar to that obtained using spleen cells. Indeed, transfers of unfractionated marrow cells mixed with thymocytes filtered over negatively charged glass bead columns resulted in reduced responses to DNP on the negatively charged carrier, whereas the results obtained using mixtures of unfiltered marrow cells and glass bead-filtered thymocytes in recipients immunized with the basic DNP-912 (T,G,L) copolymer were indistinguishable from those in which unfractionated thymocytes were transferred (Table II). These observations were verified by thymocyte fractionation over positively charged poly-L-lysine-coated columns in which case a reduction in responses was obtained only in recipients immunized with the positively charged conjugate of DNP (Table II). It is not known whether the thymocytes separated on the basis of charge belong to the same class of thymus-derived cells which appear to recognize the specificity properties of immunogens (40, 42, 43), or whether this is a distinct population of helper cells. The results reported here have demonstrated that it is possible to distinguish between thymocytes on the basis of their capacity to react with more acidic or more basic surfaces.

In contrast to the effects of "charge fractionation" on the response potential of spleen and thymocytes, no reduction was observed in the responses to DNP on either carrier after transferring unfiltered thymocytes mixed with marrow cells passed over columns of either charge (see Table III). A bone marrow-

derived population of immunocytes is responsible for the synthesis of specific antibody (18). The antibodies elicited by DNP-901 (T,G,L) and DNP-912 (T,G,L) differ in their net electrical charge, which is reflected in the light chains (44). Therefore, one might have expected that marrow cell fractionation would have affected the anti-DNP responses similarly to that observed by thymocyte fractionation. Our failure to demonstrate a correlation of the net charge phenomenon with marrow cells might have been due to technical aspects of this approach, or it could be that marrow-derived lymphocytes are not relevant for recognition of charge properties of antigens. The possibility that immature marrow precursors acquired charge recognition properties in the peripheral lymphoid compartment was excluded, since marrow cells passed through thymectomized, irradiated, intermediate hosts were not affected by the filtration. It remains to be seen whether other techniques of cell separation on the basis of charge, e.g. free flow electrophoretic separation (45, 46), will reveal a role for marrow-derived cells in the net charge phenomenon. However, the possibility exists that marrow cells acquire the ability to synthesize antibodies with charges inversely related to those of the immunogens by transfer of some factor(s) from the thymus-derived cells. Soluble factors from T cells have been shown to modify the antibody responses of marrow-derived cells (47-50). It will be of interest to establish whether the inverse relationship between the net charge of immunogens and the antibodies elicited will be demonstrated for thymus-independent antigens.

SUMMARY

An inverse relationship exists between the net electrical charge of immunogens and the antibodies they elicit (1). Results of an earlier study have demonstrated that the net charge phenomenon has a cellular basis, since the immune response potential of murine spleen cells to 2,4-dinitrophenyl (DNP) on a negatively charged synthetic polypeptide carrier was reduced by cell fractionation over negatively charged glass beads, whereas the response to the same hapten on a positively charged carrier was unaffected (14). To verify that the net charge correlation is expressed at the cellular level, spleen cells were fractionated over positively charged poly-L-lysine-coated glass bead columns, and their immunocompetence to DNP on positively and negatively charged carriers was tested by cell transfers in irradiated recipient mice. In this case, the fractionated cells showed reduced response potential to DNP on the positively charged carrier only. Thus, the cellular basis of the net charge phenomenon has been demonstrated for both positively and negatively charged immunogens (for the same specificity) by cell separation techniques over columns of opposite charge.

In order to establish whether the cell population relevant for the charge properties of immunogens was of thymus or marrow origin, thymocytes and bone marrow cells were selectively passed over positively or negatively charged

columns and mixed with unfractionated cells of the complementary type. Transfers of the filtered and unfiltered cell mixtures in irradiated recipient mice immunized with DNP on either a positive or a negative synthetic polypeptide carrier indicated that fractionation of thymocytes, but not of marrow cells, correlated with the spleen population. Thus, thymocytes fractionated over negatively charged columns and mixed with unfractionated marrow cells exhibited reduced response to DNP on the negative carrier, but normal responses to DNP on the positive carrier. The opposite result was obtained when thymocytes were passed over positively charged columns. No effect on the anti-DNP response was detected by filtration of bone marrow cells over columns of either charge. These findings indicate that it is possible to distinguish between thymocytes on the basis of their capacity to react with more acidic or more basic surfaces and that a population of thymus-derived cells may recognize immunogens on the basis of their overall electrical charge. No evidence was found by these techniques that marrow-derived cells contribute to the net charge phenomenon.

The authors wish to express their gratitude to Mrs. Heidi Zinger and Mrs. Etti Ziv for excellent technical assistance.

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