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I am submitting herewith a dissertation written by Richard Lee Krogsrud entitled "Age-Related Decline in Two Parameters of the Humoral Immune Response: B Cell Proliferation and T Cell Helper Function." I recommend that it be accepted in partial fulfillment of the requirements for the degree of Doctor of Philosophy, with a major in Biomedical Sciences.

Eugene H. Perkins, Major Professor

We have read this dissertation
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AGE-RELATED DECLINE IN TWO PARAMETERS OF THE HUMORAL IMMUNE RESPONSE: B CELL PROLIFERATION AND T CELL HELPER FUNCTION

A Dissertation

Presented for the

Doctor of Philosophy

Degree

The University of Tennessee

Richard Lee Krogsrud

December 1975

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To my wife, Winnie,

without whom I would not be here,

and

In memory of my father, Olaf,

without whom I would not be.

ACKNOWLEDGMENTS

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ABSTRACT

This investigation was an inquiry into the cellular nature of the well documented decline in humoral immunity of aging mice based on (1) the capacity of the antigen-stimulated B cell precursors of antibody forming cells to proliferate, and (2) the capacity of T cells to regulate the above proliferation of B cells.

The proliferative response of B cells to the mitogens LPS and Sepharose-bound Con A were tested in microculture, assaying the synthesis of DNA by measure of the incorporation of tritiated thymidine. The response of old B cells to LPS was found to be only one half that of young adult cells. In addition, a component of the splenic response to LPS was found to be eliminated by anti-theta serum treatment while the response to Sepharose-bound Con A was completely eliminated.

The helper function of T cells was determined in a cell transfer system using hapten-primed (DNP-HGG) spleen cells (depleted of theta-bearing cells) from young adult mice, in combination with carrier-primed (BGG) cells (depleted of IgM-bearing cells) from a range of age groups over the entire adult lifespan. T helper cells from old animals were found to be deficient in the capacity to stimulate both proliferation (i.e., expansion of DNP-sensitive B cell clones) and differentiation (i.e., causing the switch from precursor to antibody-secreting cells).

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I. INTRODUCTION

T and B Cell Interaction

The concept of interaction between two or more cell types in the development of the humoral immune response, although now well established, began with the demonstration by Claman, Chaperon and Triplett (1) that lymphocytes from the thymus cooperated with lymphocytes from bone marrow to produce antibody forming cells. These cells have since become designated T cells (thymus-derived) and B cells (bone marrow-derived). Davies et al., (2) subsequently showed, by combining chromosomally marked bone marrow cells from CBA-T6 mice with thymus cells from normal CBA mice, that the B cells were the precursors of antibody forming cells. Hence the T cells were tagged as auxiliary or "helper" cells, since they were needed to produce antibody responses but did not manufacture antibody themselves.

A third cell type was implicated in this cooperative immune response by the *in vitro* work of Mosier and Coppleson (3), who allowed spleen cells to adhere to petri plates and then removed the nonadherent cells. When these populations were tested separately, they produced very few antibody forming cells. However, when they were combined the response was restored to normal levels. Among the adherent cells were the phagocytic mononuclear cells (macrophages).

How these cell types interact still remains to be established. However, a significant clue was the discovery by Mitchison (4) of the "carrier effect." His observations were based on a type of artificially prepared antigen which consists of a protein "carrier" chemically

conjugated with distinct chemical moeities or "haptens." A pertinent example would be the hapten, 2,4-dinitrophenol or DNP. If animals are primed with the DNP hapten on a certain carrier, e.g. bovine gamma globulin (DNP-BGG), and later challenged with the same hapten on a different carrier, e.g. human gamma globulin (DNP-HGG), the immune response specific for DNP is much smaller than if the challenge were made with DNP on the original carrier (DNP-BGG). Combining the hapten-primed cells with carrier-primed cells was subsequently shown to be the only way to get a good response when the hapten-carrier conjugate is used as the challenge antigen. Raff (5) identified the carrier reactive cells as thymus-derived by using an antiserum specific for T cells (anti-theta serum).

Although the T cell is necessary in the response to many antigens, there is a class of T-independent antigens which are capable of directly stimulating B cells to become antibody forming cells (6-17). However, it is important to note that in all of these responses, few, if any antibody forming cells produce immunoglobulins other than IgM (195). This indicates that T cell helper function may be necessary in the activation of the IgG producing cells.

It is also evident from the work of Coutinho and Moller (18), and Andersson et al. (19, 20), that several of these T-independent antigens are really "mitogenic" in nature. This means that they stimulate lymphocytes to proliferate in the absence of specific antigen. Bacterial lipopolysaccharide (LPS) is one of these mitogens which seems to be stimulatory only toward B cells. Interestingly, the active

component of LPS from E. coli can be purified and it has been shown to have all the mitogenic activity but none of the antigenic specificity of the whole molecule (21). The use of such reagents has provided additional clues to the mechanism of B cell stimulation (discussed in 22).

T cells are also responsive to mitogen stimulation, but seem to have slightly different requirements, since the plant lectins phytohemagglutinin (PHA) and Concanavalin A (Con A) stimulate only T cells (23, 24) unless they are bound to an insoluble support such as a plastic petri plate or Sepharose (25-27), in which case they stimulate only B cells. Studies with mitogens and T-independent antigens have lent support to several theories of T and B cell interaction (22). These theories relegate the T cell to a role of (a) completely nonspecific stimulation (Coutinho, 28 and Moller, 29) which seems unlikely in light of the carrier effect; (b) antigen presentation, either directly to the B cell (Mitchison, 4; Bell, 30) or indirectly via the macrophage (Feldman, 31); (c) production of a second signal which is either specific (Barton and Diener, 32; Bretscher, 33; Dutton, 34; Sachs and Dickler, 35) or nonspecific (Nossal and Schrader, 36; Schimpl and Wecker, 37; Talmage and Thomas, 38; Weigle and Skidmore, 39) or (d) influencing the macrophages to produce soluble factors (Weigle and Skidmore, 39; Moller, 29) or to phagocytose the bound antigen from the B cell surface (Mitchell, 40, 41).

Evidence is mounting in several laboratories (42-44) which indicates that the T cell may mediate both the proliferative and differentiative events of B cell stimulation as separate functions.

Thus, North and Feinstein (42) showed that both the number and size of hemolytic foci in irradiated spleens of animals receiving antigen and a constant number of B cells increased with the number of T cells added. Hunter and Kettman (43) showed that supernatants from mixed lymphocyte cultures (T cell-produced soluble factors) had a dialyzable fraction which only expanded the clones of B cells which had already been stimulated by a nondialyzable fraction.

Although the mechanism of T and B cell interaction is still in doubt, the perturbations of the humoral immune system introduced through senescence may yield insight into this problem. The research presented within does this in addition to providing evidence of the effect of aging.

Age-Related Changes in Humoral Immunity

The humoral immune system of the mouse provides an excellent model system both for the study of cellular interactions and, since the life span is only a few years, for the study of aging. A systematic study of age-related changes in the immune system of mice was first undertaken by Makinodan and Peterson (45, 46) who showed significantly decreased antibody formation in old mice in response to sheep erythrocyte antigens. Since then, numerous studies have confirmed the decline of humoral immunity in this and other aging systems (reviewed in 47, 48 and 49).

The consideration of environmental factors of old mice as being responsible for defective immune responses has led to the minimization

of conditions external to the cells themselves (50, 51). By transferring young mouse spleen cells into old or young lethally irradiated mice, these authors showed that old environments could support up to 90% of the immune activity found in young control mice.

Of the three cells known to be involved in humoral immunity, the macrophage has been absolved of any age related loss of phagocytic function (52, 53). In addition, the results of Heidrick and Makinodan (51) clearly demonstrate that old adherent cells are equally able to support the in vitro response of nonadherent cells as in the Mosier-Coppleson system (3).

A characterization of age-related changes in T and B cell compartments has been carried out by Price and Makinodan (54) who began to explain the twenty-fold decrease in the primary plaque forming cell response to sheep erythrocytes by showing differences in the frequency of antigen-sensitive B cells (a 3.5-fold decrease) and antigen-sensitive T cells (a 2-fold increase) in old spleens. They also calculated a twenty-fold decrease in the immunological "burst size," or, in other words, the number of progeny from one immunocompetent unit. This indicated either a deficiency in B cell proliferation or in the interaction between T and B cells (55).

The use of mitogens to stimulate the proliferation of T and B cells has demonstrated a tremendous (90%) lack of responsiveness in old T cells (56, 57) and a small deficiency in B cells (20-60%) (52-58). The former studies were carried out with

Phytohemagglutinin and the latter with PPD (purified protein derivative from tuberculin) or bacterial lipopolysaccharide (LPS).

In light of experiments by Elfenbein and Gelfand (59) the mitogen studies of T cell proliferation may include a significant B cell component. Conversely, the B cell proliferation in response to PPD and LPS may be partially T cell dependent. Thus, the proliferative defects in old animals may not be as formerly described. This possibility was tested for B cells in the first section of this dissertation by using LPS and Sepharose-Con A, both presumably B cell mitogens, in the presence or absence of T cells.

Still lacking in the picture of the immune response and aging is evidence concerning the interactions of T and B cells. The second part of this dissertation, therefore, deals with the possibility of defective helper T cell function in senescing mice by making use of the carrier effect (4). Accordingly, T cells from carrier-primed animals of different ages were combined with B cells from hapten-primed animals and injected into irradiated recipients along with the hapten-carrier conjugate. The carrier-reactive helper T cells were thus able to be compared on the basis of their capacity to aid the response of a standard B cell population.

II. B CELL PROLIFERATION

Materials and Methods

Mice. Female BC3F1 mice were purchased from Cumberland View Farms, Clinton, Tennessee at about five weeks of age were maintained in a conventional animal facility. "Young" mice were 3 to 7 months of age. "Old" mice were free of pathology, as judged by careful macroscopic examination of the internal anatomy, and ranged from 28 to 32 months of age.

Mitogens. Lyophilized E. coli 055:B5 lipopolysaccharide (LPS) (Difco Laboratories, Detroit, MI) and lyophilized Concanavalin A (Con A) (Sigma Chemical Co., St. Louis, MO, Lot #101C-5390) were reconstituted with RPMI 1640, (GIBCO, Grand Island, NY) passed through a sterile 0.22 micron Millipore filter (Millipore Corp., Bedford, MA) and stored at -60°C. Lyophilized, purified phytohemmagglutinin (PHA) (Wellcome Research Laboratories, Beckenham, England) was reconstituted with sterile saline. Con A bound to Sepharose (8 mg. Con A per ml. of packed Sepharose) was obtained from Pharmacia Chemicals, Uppsala, Sweden, washed several times, centrifuged and dialyzed for 36 hours against three changes of phosphate buffered saline.

Antisera. Anti-theta serum was prepared by injecting AKR/J mice intraperitoneally at weekly intervals with about 50 x 10^6 thymocytes from young adult C3H/HeJ mice (both strains obtained from Jackson Laboratories, Bar Harbor, ME). The mice were bled from the

retroorbital sinus on day six after the eighth injection. The blood was allowed to clot and the serum was removed after centrifugation and absorbed with BC3F1 bone marrow and erythrocytes (equal volumes, three times). Titration showed that the anti-theta serum gave optimal killing (i.e., maximal specific killing vs. minimal killing of controls) for thymocytes (90%) at a dilution of 1/50. Complement and normal serum controls yielded less than 10% dead cells, as judged by trypan blue exclusion.

Anti-immunoglobulin M serum was a gift from S. Durum and Dr. N. Gengozian of Oak Ridge Associated Universities. It was prepared in rabbits using mouse IgM conjugated to sheep red blood cells. The serum was absorbed with mouse liver cells, red blood cells and thymus cells. Killing of spleen cells was 50% at a dilution of 1/50. This dilution also gave a maximal reduction (80-90%) in the LPS mitogenic response (B cell specific) while the PHA response (T cell specific) was greater than 80% of the control (normal serum and complement treated spleen cells).

Cell suspensions. Spleen cell suspensions were prepared by teasing spleens apart and washing the cells free with ice cold RPMI 1640 culture medium using a 22-gauge needle attached to a syringe and forceps. The culture medium was buffered with Hepes and contained 10 units per milliliter of penicillin and 10 micrograms per milliliter of streptomycin sulfate. Thymus cells were prepared in the same manner. Bone marrow plugs were forced out of femurs with RPMI 1640 using a 22-gauge

needle and a 5 ml. syringe. All cell suspensions were dispersed by repeated aspiration through the needle and then passed through sterile wire screen of 200 mesh (Cambridge Wire Cloth Co., Cambridge, MD) to eliminate tissue debris. The cells were washed by diluting in 15 ml. of medium and spinning down for 10 minutes at 800 rpm (150 x g) in an IEC model PR-2 refrigerated centrifuge. Cells were resuspended in the culture medium and nucleated cell counts were made in a hemocytometer using Turk's solution.

Treatment of cells with antisera. Spleen cells (10^7 per ml.) were incubated in Hank's balanced salt solution containing the appropriate concentration of antiserum for 30 minutes at 4°C. Then guinea pig complement (Colorado Serum Co., Denver, CO) which had been absorbed with agar (10 mg./ml.) to eliminate nonspecific cytotoxic factors was added to make a 1/10 dilution. The cells in suspension were incubated for another 30 minutes at 37°C., centrifuged and resuspended in RPMI culture medium.

Culture conditions. Cell concentrations were adjusted to 3.33 x 10^6 cells per milliliter based on counts made before antiserum treatment. Spleen cells (0.15 ml) were distributed into the wells (5 x 10^5/well) of Microtest II tissue culture plates (Falcon Plastics, Oxnard, CA), followed by 0.025 ml. of mitogen solution or culture medium alone, using an Oxford Sampler (Oxford Instruments, San Mateo, CA). After one hour of incubation in a 5% CO_2 atmosphere at 37°C., 80% humidity, the cultures were fed 0.025 ml. of fetal calf serum (Reheis Chemical Co.,

Phoenix, AZ). Twenty-four hours later, 2.5 microCuries (5 Curies/millimole) of ^{3}H-TdR (Amersham/Searle, England) were added to each well and the plates again incubated for 24 hours.

Harvest of cultures. Using an automatic cell culture harvesting apparatus (Otto Hiller Co., Madison, WI), the culture wells were aspirated, rinsed twice with 0.2 ml. of saline and the solution was passed through a filter strip on which the DNA was precipitated by 10% trichloroacetic acid, rinsed with 95% ethanol and dried for several hours in a 100°C. drying oven. The filters were placed in 15 x 45 mm. glass shell vials (Owens-Illinois, Toledo, OH). One milliliter of Liquifluor/toluene counting solution (42 ml./liter) (New England Nuclear, Boston, MA) was added to each vial. Samples were counted in a Beckman model LS-230 liquid scintillation counter.

Results

Mitogen dose-response kinetics. The peak of DNA synthesis as revealed by tritiated thymidine incorporation in response to B cell mitogen, LPS, occurred within the second 24 hours of culture for both young and old spleens. Therefore, the dose-response experiments were carried out under the culture conditions described in the previous section. The labeling with ^{3}H-TdR took place during the final 24 hour period of culture (24-48 hrs.).

The curves for LPS, Sepharose-Con A and soluble Con A are represented in Figures 1, 2, and 3, respectively. In each case, the optimal doses

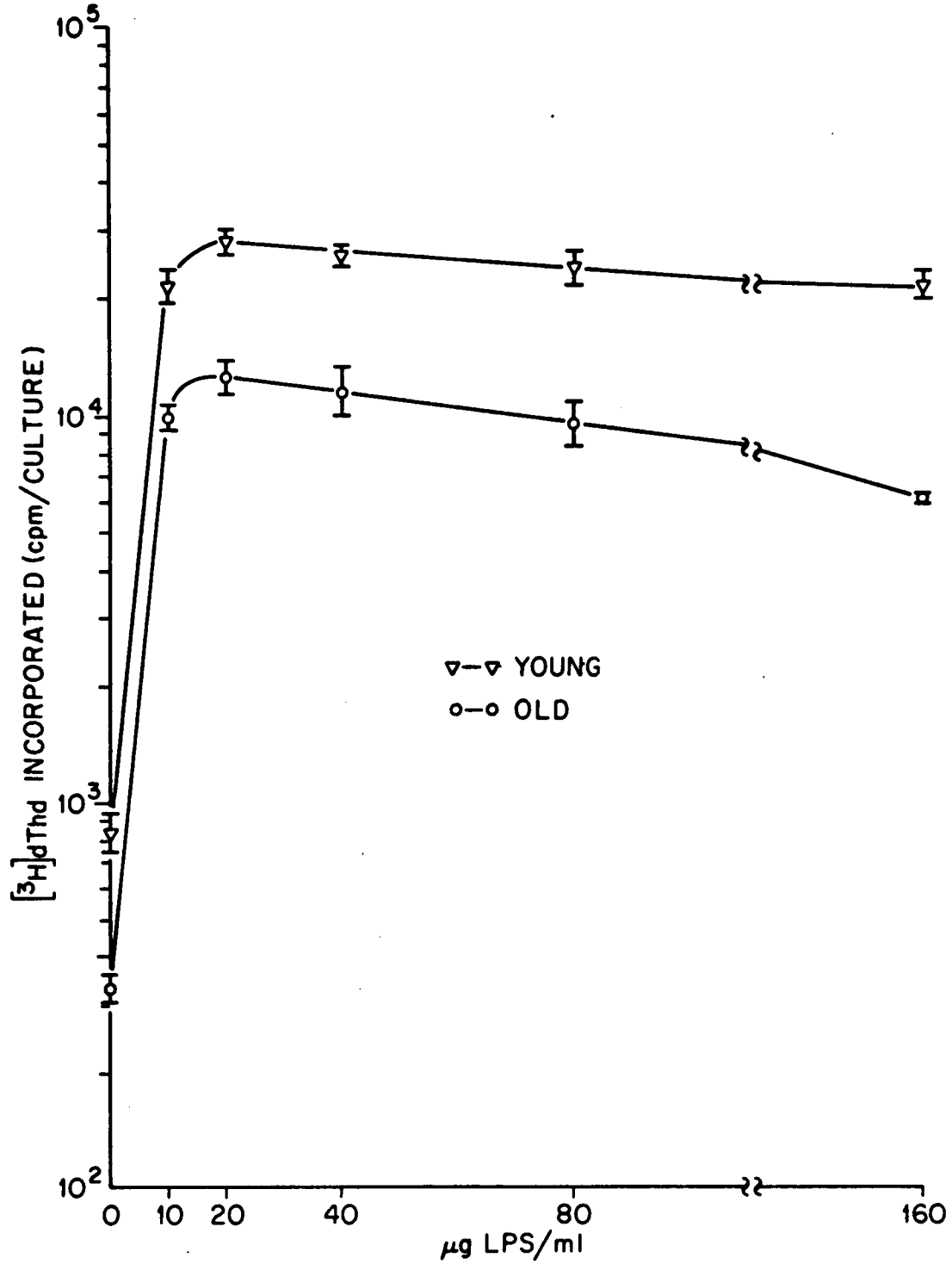

Figure 1. In vitro incorporation of ^{3}H-thymidine into DNA of LPS-stimulated "old" or "young" spleen cells.

Four replicates of 5 x 10^5 cells from pools of three "young", (3-months, ▽) or "old," (33-months, O) spleens were incubated at 37°C for 48 hours with various doses of LPS and labelled with ^{3}H-thymidine during the second 24 hours of culture. Vertical bars represent 95% confidence limits.

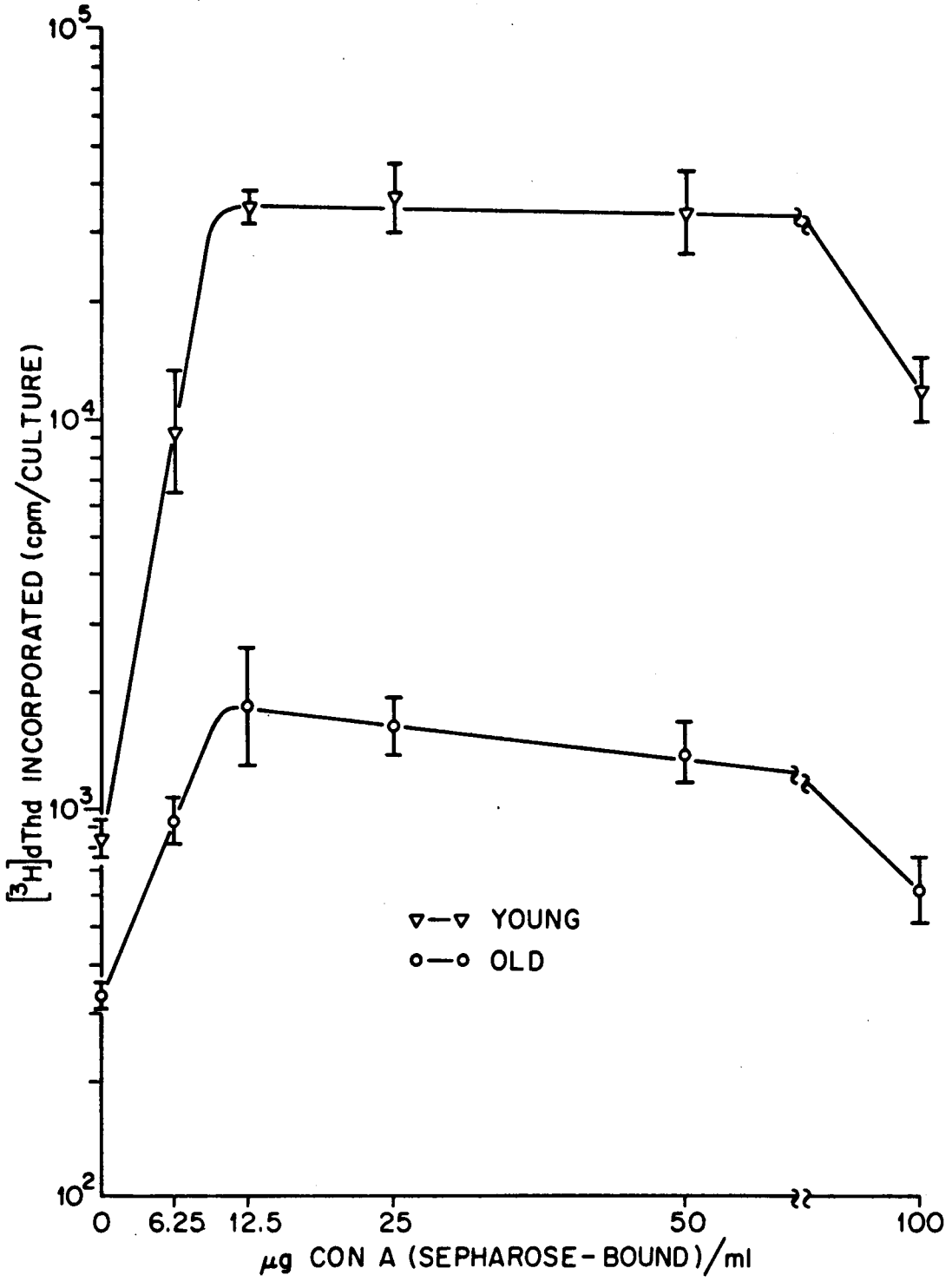

Figure 2. In vitro incorporation of ^{3}H-thymidine into DNA of Sepharose-bound Con A stimulated "old" or "young" spleen cells.

Four replicates of 5 x 10^5 cells from pools of three "young," (4 months, ▽) or "old," (30 months, O) spleens were incubated at 37°C for 48 hours with various doses of Sepharose-Con A and labelled with ^{3}H-thymidine during the second 24 hours of culture. Vertical bars represent 95% confidence limits.

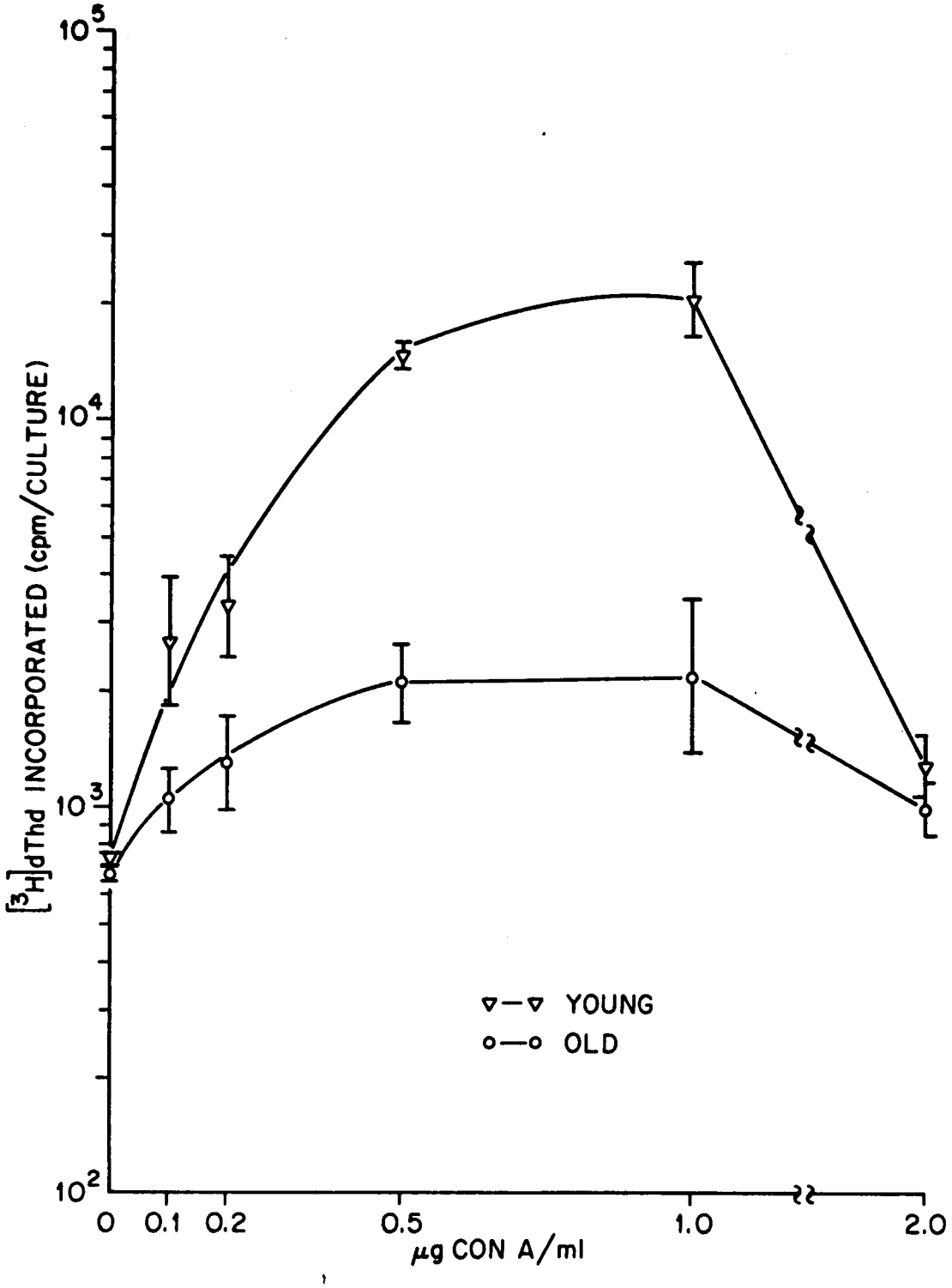

Figure 3. In vitro incorporation of ^{3}H-thymidine into DNA of Con A stimulated "old" or "young" spleen cells.

Four replicates of 5 x 10^5 cells from pools of three "young," (4 months, ▽) or "old," (30 months, O) spleens were incubated at 37°C for 48 hours with various doses of soluble Con A and labelled with ^{3}H-thymidine during the second 24 hours of culture. Vertical bars represent 95% confidence limits.

and general shapes of the curves are similar for both age groups. All three mitogens exhibit fairly broad ranges for maximal response. Young and old responses differed only in magnitude. Thus for LPS there was a 2-fold higher incorporation in the young spleen cells while there were 23 and 9-fold higher responses for Sepharose-Con A and Con A, respectively.

Effect of anti-theta serum treatment. Since the purpose of these experiments was to establish the possibility of a B cell proliferative defect, it was important to make certain that LPS and Sepharose-Con A were indeed T cell independent (9, 12-14, 18-20, 25-27). Therefore, old and young spleen cell suspensions were divided into two portions and treated with either AKR anti-theta serum or normal AKR serum. The cells were then incubated with complement to destroy theta-bearing T cells.

Table I shows that the killing of theta-positive cells eliminated the response to both bound and soluble forms of Con A. In contrast, the response to LPS, while still significant, was reduced by 50% in young and 45% in old spleen cells. Viability counts on both anti-theta treated populations were about 55%, while normal serum controls remained 75% viable.

Figure 4 shows the LPS dose-response curve of anti-theta treated young and old spleen cells. In this experiment, the reduction was again 50% for young but only 20% for old spleen cells.

To eliminate the possibility that B cells were being affected by the antiserum, bone marrow was taken from young and old femurs, treated with

TABLE I

THE EFFECT OF ANTI-THETA SERUM ON MITOGEN RESPONSES OF YOUNG OR OLD SPLEEN CELLS

Age (months)	Treatment[e]	Incorporation of ^{3}H TdR into DNA (cpm/culture)[a]			
		LPS[b]	Sepharose-Concanavalin A[c]	Soluble Concanavalin A[d]	No Mitogen
4	N-AKR	22,829± 608[f]	31,548±9,404	20,050±1,969	1,006±72
4	Anti-Theta	11,295±2,044	986± 27	844± 21	833±31
30	N-AKR	7,300± 309	1,732± 295	2,353± 209	998±32
30	Anti-Theta	3,901± 939	1,103± 81	1,114± 141	1,113±75

[a]Incorporation into 5 x 10^5 cells/culture (based on counts made before treatment), during exposure to label between 24 and 48 hours of culture.

[b]*E. coli* 055:B5 lipopolysaccharide (20 µg/ml).

[c]Concanavalin A bound to sepharose (25 µg/ml).

[d]Concanavalin A in soluble form (1 µg/ml).

[e]Cells were incubated with either normal AKR serum or anti-theta-C3H serum for 30 minutes at 37°C followed by 30 minutes at 37°C with a 1/10 dilution of guinea pig complement.

[f]Standard error of the mean for four replicate cultures.

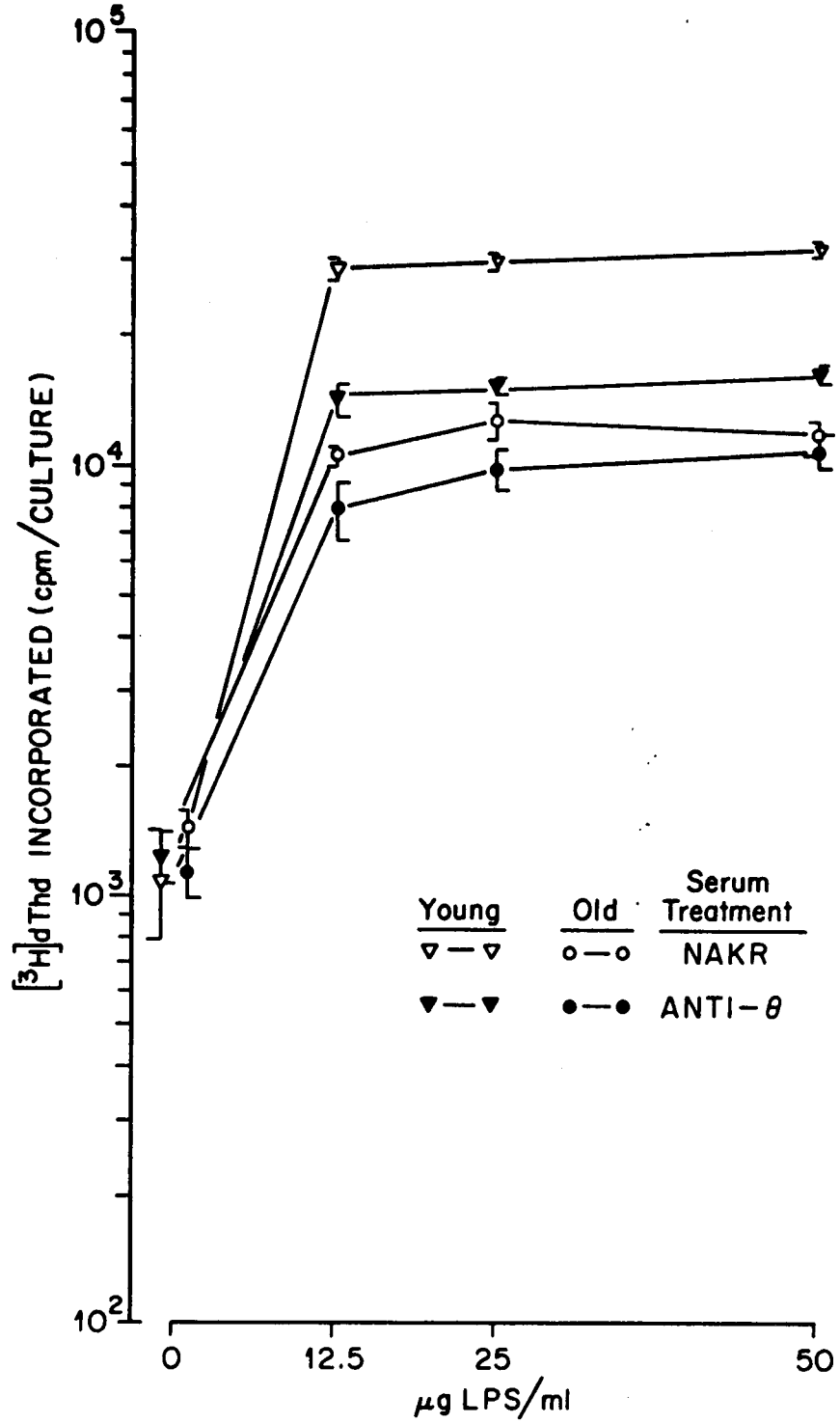

Figure 4. In vitro incorporation of ^{3}H-thymidine into DNA of LPS-stimulated spleen cells: Effect of anti-theta serum treatment.

Four replicates of 5 x 10^5 cells from pools of three "young," (4 months, ∇, ▼) or "old," (30 months, O, ●) spleens, treated either with normal AKR serum (∇, O) or anti-theta (C3H) serum (▼, ●) for 30 minutes at 0°C followed by 30 minutes at 37°C with agar-absorbed guinea pig complement, were cultured for 48 hours with various doses of LPS and labelled with ^{3}H-thymidine during the second 24 hours of culture. Vertical bars represent 95% confidence limits.

anti-theta serum and cultured as in the previous experiments. The results (Table II) bring out two important points: (a) The antiserum has negligible effect on the LPS response of either young or old bone marrow but it does reduce the PHA stimulation to background levels; (b) there is no age-related change in the bone marrow blastogenic response to LPS. Note also that the background (nonstimulated) level of DNA synthesis is considerably higher in bone marrow than in spleen, reflecting a level of ongoing hematopoesis in the former tissue. On the other hand, splenic B cells seem to have a greater capacity to respond to the mitogen.

Discussion

Based on these data from *in vitro* blastogenesis of spleen cells in response to *Escherischia coli* 055:B5 lipopolysaccharide, the aged mouse has a small but significant defect in the splenic B cell compartment. The source of the defect may be related to (a) fewer cells responding to LPS, (b) fewer divisions per cell, (c) differential cell death in the presence of the mitogen, or (d) an undetermined cellular cooperative effect.

The third possibility is improbable since trypan blue exclusion tests after culturing show that although only 50% of the original cells are still alive, both young and old cell cultures had similar viability. These results are in accord with the observations of Hung et al., (57) using PHA in petri dish cultures. The relative proportion of T and B cells, however, was not determined after harvest of the cells. Data from Perkins et al., (60) indicate a 10% increase in theta-positive (T)

TABLE II

EFFECT OF ANTI-THETA SERUM ON MITOGEN RESPONSES OF YOUNG OR OLD BONE MARROW OR SPLEEN CELLS

Age	Tissue	Treatment[b]	Incorporation of ^{3}H-TdR into DNA (cpm/culture)[a]			
			LPS[c]	PHA[d]	No Mitogen	Percent Viability Following Treatment
4	Bone Marrow	N-AKR	11,992±1,303[e]	15,030±4,271	6,994± 986	80
4	Bone Marrow	Anti-Theta	10,628±1,903	8,072±1,665	7,196±1,219	75
4	Bone Marrow	None	18,199±3,306	NT[f]	5,109±1,204	90
29	Bone Marrow	None	16,961±1,880	NT	3,777± 534	90
4	Spleen	N-AKR	25,839±2,663	50,778±2,793	980± 117	80
4	Spleen	Anti-Theta	12,019± 838	6,441± 999	667± 75	55
4	Spleen	None	30,877±2,429	NT	2,130± 176	NT
29	Spleen	None	15,267±1,083	NT	1,391± 133	NT

[a]Incorporation into 5 x 10^5 cells/culture (based on counts made before treatment) during exposure to label between 24 and 48 hours of culture.

[b]Cells were incubated with either normal AKR serum or anti-theta-C3H serum for 30 minutes at 0°C followed by 30 minutes at 37°C with a 1/10 dilution of agar absorbed guinea pig complement.

[c]*E. coli* 055:B5 lipopolysaccharide (25 µg/ml).

[d]Phytohemagglutinin (6.25 µg/ml).

[e]Standard error of mean for four replicate cultures.

[f]Not tested.

cells of old spleen compared to young adult, while immunoglobulin-bearing (B) cells decrease by 5-7%. However, these minimal differences in ratios of T and B cells appear insufficient to account for the defect seen in the blastogenesis data.

Although the B cell probably goes through a number of divisions in the course of an immune response, (61, 62), it is not clear whether each division requires the restimulation of the cell by antigen or other factors. The same question applies to the mitogen stimulation. During the 48-hour period of culture used in these experiments, it is not clear how many divisions a single cell might undergo. Sado and Makinodan (63) estimated that the cell cycle of antigen stimulated blast cells of the rabbit in a secondary response is from 8 to 9 hours. In mice, the generation time may be 12 to 14 hours (64). Since the first DNA synthesis in LPS-stimulated cultures occurs 12 to 24 hours after initiation (65), time would allow for one or two cell cycles during the labeling period. Thus, the number of cells acquiring label may be two to four times the original responding number. If so, then the effect of aging may be interpreted as a decrease in the ability to proliferate or an increase in the generation time.

Gunther et al., (24) in studies using Con A (primarily a T cell mitogen), found the numbers of autoradiographic grains per cell did not change over a 72 hour period, while the percentage of blast cells and the percentage of labeled blast cells increased in parallel to the ^{3}H-Thymidine incorporation. This means that new cells were responding to the mitogen with time and they, once stimulated, underwent only one

division. Hung et al., (57) found that, whereas the total incorporation of ^{3}H-TdR into young spleen cells was ten times that of the old mice, the number of dividing cells (labeled blast cells) differed by only a factor of four. If these observations hold for LPS-stimulated cultures, then the responding compartment of old animals may be only one half that of younger mice. In the report of Janossy et al., (66) using young adult mice, the fraction of responding B cells was estimated to be 20 to 30% for LPS stimulated splenic B cells. Thus, in the present study, only 10 to 15% of old B cells may be responding. This interpretation would be in agreement with the 3.5-fold decrease in the frequency of antigen responsive cells calculated by Price and Makinodan (54). The data here are similar to the results of Makinodan and Adler (55) and Gerbase-deLima et al., (58), however these investigators noted a 20% to 70% decrease in the LPS response of 24 and 30 month old mice, respectively, as compared to young adult mice.

As expected, following treatment with anti-theta serum the reduction of the Con A response of young or old spleen cells was complete (i.e., reduced to the level of nonstimulated controls). However, the complete reduction in the case of Sepharose-Con A was an unexpected result. According to Andersson and coworkers (26, 27), locally concentrated Con A is stimulatory for B cells, contrary to the mitogen's normal specificity for T cells. This reversed cellular specificity was also noted for locally concentrated PHA (25). The data presented here deny the T cell independence of the Sepharose-Con A mitogenic response. That is, the elimination of T cells from the

population also eliminated the mitogenic response. Possibly the explanation for this lies in the differences in the density of molecules needed for B cell stimulation. According to Mitchison's "matrix" model (4), the T cell should be stimulated by a lower density of antigenic determinants and then serve as a concentrating device to stimulate the B cells. Unfortunately, the actual density of Con A available for binding responsive cells in these experiments is nearly impossible to calculate because of the porous structure of the Sepharose polymeric beads.

The reduction of LPS responses as a consequence of anti-theta serum treatment might be explained by (1) nonspecific cytotoxicity of the anti-theta serum for B cells, (2) T cell response to the mitogen, or (3) potentiating effects of T cells. The first explanation seems unlikely since both viability and LPS responses of anti-theta treated bone marrow suspensions were not significantly different from normal serum controls. The second suggestion is contrary to most published data (12-14, 18-21). The third possibility has been suggested by several investigators in regard to the potentiation of polyclonal activation of antibody production, (68-72). That is, these authors have observed that LPS-stimulated specific antibody production to several noncross-reacting determinants is increased when T cells are present. Direct activation of T cells was ruled out in their experiments and nonspecific factors produced by T cells that enhanced the activity of B cells were implicated. In addition, Elfenbein and Gelfand, (59) have evidence that mitogen (Con A or PHA) coated T cells may stimulate B cells. In this

case, however, the role of soluble factors was minimized. Assuming that the polyclonal activation by lipopolysaccharide includes both proliferative and differentiative signals (reviewed in 22 and 73, 74), a similar T-dependence of the blastogenic response would be expected.

It seemed plausible, based on (1) the arguments presented above, (2) the anti-theta insensitivity of young and old bone marrow responses to LPS, and (3) the differential sensitivity of young and old splenic LPS responses, that the addition of young T cells might restore the response of old spleen cells to the level of young adult mice. However, experiments using either thymus or anti-immunoglobulin M treated spleen as sources of T cells have given only equivocal results.

In summary, then, the data present evidence of a relatively small but significant proliferative defect in the B cell response to E. coli lipopolysaccharide. Contrary to expectation, however, both young and old LPS responses seemed to have a T cell dependent component. In relation to the overall decline in the humoral immune response of aging animals, this B cell proliferative defect seems to play only a minor role. Therefore, the investigation of T cell regulatory functions may prove more productive.

III. T CELL HELPER FUNCTION

Materials and Methods

Mice. Female BC3F1 mice were purchased from Cumberland View Farms, Clinton, Tennessee at about five weeks of age and maintained in our conventional animal facility. "Young" mice were 3 to 7 months of age. "Old" mice were free of pathology, as judged by careful macroscopic examination of the internal anatomy, and ranged from 14 to 34 months of age.

Preparation of antigens. Dinitrophenylated bovine and human gamma globulins were prepared according to the method of Eisen (75). Briefly, 0.5 gm of bovine or human gamma globulin (fraction II from plasma, Pentex, Inc., Kankakee, IL) was dissolved in 25 ml. of saline and, after addition of 0.5 gm of sodium dinitrobenzene sulfonate (Eastman Kodak Co., Rochester, NY), the pH was adjusted to 10.0 -10.5 with sodium carbonate. This solution was agitated overnight at room temperature. The conjugated protein was then precipitated with ethanol (8 volumes), washed several times and dialyzed against phosphate buffered saline until no yellow color appeared in the dialysate. From the optical densities of samples dissolved in 0.1 N NaOH, the number of hapten groups per molecule of carrier was found to be 19 for bovine gamma globulin ($DNP_{19}BGG$) and 14 for human gamma globulin ($DNP_{14}HGG$).

BGG, $DNP_{19}BGG$ and $DNP_{14}HGG$ were adsorbed to 325 mesh Bentonite (E. H. Sargent and Co., Chicago, IL) according to the method of Gallily

and Garvey (76). The amount of antigen adsorbed was estimated from the optical density of the supernatant (by difference). Suspensions were adjusted to about 1 mg. bound antigen per ml.

Priming of donors. Mice were given, intraperitoneally, 0.2 ml. of a 1 mg./ml. suspension of antigen adsorbed to bentonite. Carrier-primed (BGG) donors were used 2 weeks after injection (4, 77, 78) except as noted, while hapten-primed (DNP-HGG) donors were used 4 to 6 weeks after priming.

Preparation of spleen cell suspensions. Donor spleens were excised and teased apart in Hanks' balanced salt solution (HBSS) (GIBCO, Grand Island, NY) with forceps and a syringe having a 22-gauge needle. Cells were passed through a 200 mesh stainless steel wire screen, washed once in 15 ml. of HBSS, spun down at 150 x g in a refrigerated centrifuge and resuspended in HBSS at 10^7 cells per ml. prior to treatment with antisera.

Antiserum treatments. Hapten-primed (DNP-HGG) spleen cells were treated with a 1/40 final dilution of anti-theta serum at $4^{o}C$ for 30 minutes, followed by a second 30 minute incubation at $37^{o}C$ with a 1/10 final dilution of agar-absorbed guinea pig complement. Carrier-primed (BGG) spleen cells were treated with a 1/50 dilution of anti-immunoglobulin M serum (anti-IgM) under the same conditions. Following complement lysis of the sensitive cells, the suspensions were washed and resuspended in HBSS containing 10 units/ml. of penicillin-streptomycin (GIBCO, Grand Island, NY) and 5 units/ml. of Heparin

(Philadelphia Laboratories, Inc., Philadelphia, PA). The effectiveness of the treatments was checked by counting viability of the cells in 0.1% trypan blue in saline.

Irradiation of recipients. Recipients were 2 to 4 month old female BC3F1 mice. They were exposed to 800 Roentgens of X-irradiation from a G.E. Maxitron 300 X-ray unit. The dose rate of 180 R per minute was given from 70 centimeters.

Cell transfers. The challenge antigen ($DNP_{19}BGG$ adsorbed to bentonite) at 0.1 mg./ml. was mixed with various combinations of hapten- and carrier-primed spleen cells just prior to intravenous injection into the irradiated recipients. The total volume of the injection was always 1.0 ml.

Plaque assay. The method of Silver et al., (79) with slight variations was used to detect antibody forming cells specific for the DNP hapten. Basically the method involves the following steps:

1. Raising anti-sheep erythrocyte antibody in chickens.
2. Purification of the immunoglobulin fraction by ammonium sulfate (40%) precipitation (80 and Yang, W.-K., personal communication).
3. Derivitization of the immunoglobulin as described for the preparation of antigens (see above). Preparations used in these experiments were from the same batch of chicken gamma globulin (CGG) and yielded 6 moles of DNP per mole of globulin (DNP_6CGG).

4. Preparation of target cells. Sheep red cells (10% suspension in saline) were incubated with 200 µgm./ml. of DNP_6CGG for 30 minutes at 37°C, then washed several times in saline and resuspended at 20%. Control sheep red cells were incubated in nonderivatized anti-SRBC CGG at 50 µgm./ml. This concentration gave a similar pattern of agglutination to the 200 µgm. per ml. of derivatized globulin.

5. Assay of plaque forming cells. The target or control sheep red cells were mixed with dilutions of spleen cells in warm (45°C) agarose (0.7%) (Fisher Scientific, Fair Lawn, NJ) and spread immediately on glass slides sublayered with 0.1% agarose. The slides were inverted on special plexiglass trays and agar adsorbed guinea pig complement (1/10 in Eagle's Minimal Essential Medium, EMEM) was carefully flowed underneath. After a one hour incubation at 37°C, the slides were scored for direct plaque forming cells. They were then replaced on fresh trays of EMEM containing complement and rabbit anti-mouse gamma globulin antibody (1/10) (Lot #4552, Cappell Laboratories, Downington, PA) to facilitate the production of indirect plaques. After another hour, the slides were removed, drained, fixed in acetone, cleared in water, dried and scored for indirect plaques. Plaques were counted under a dissecting microscope at 7-fold magnification.

Results

The measure of T cell helper function in these experiments is based on the interaction of "purified" populations of T cells from various age groups with an indicator population of "purified" young B cells in a young environment. T cell populations are depleted of IgM-bearing cells and B cell populations are depleted of theta-bearing cells. Thus, with T cells being the only variable, differences in the resultant plaque forming cell responses directly reflect differences in T cell function. To investigate the kinetics of these interactions, T cell populations were tested at various doses up to 25 x 10^6 cells.

Using a similar cell transfer system, Bosma et al. (81) established that when more than 20 x 10^6 spleen cells are injected into immunologically inert recipient mice, the number of plaque forming cells per increment of spleen cells (i.e., the "burst" size) is constant. Therefore cell transfers were made such that the total number of cells always exceeded 25 x 10^6.

Purity of "T" and "B" cell populations. In preliminary experiments, (Table III) the effectiveness of anti-theta serum treatment in the removal of functional T cells from hapten-primed spleen cells was demonstrated. When hapten-primed cells were incubated with a 1/50 dilution of the antiserum, both direct and indirect PFC responses were reduced by more than 85% (compare line 1 with line 2). As an added precaution, however, later experiments were carried out with a 1/40 dilution of antiserum, which was still in the optimum range.

TABLE III

EFFECTS OF ANTI-THETA AND ANTI-IMMUNOGLOBULIN SERA ON DNP-SPECIFIC PFC RESPONSE IN CELL TRANSFER[a]

"T" Cell Source	Treatment	Treatment[c] of "B" Cells	Challenge	PFC/Recipient Spleen[b] Direct	Indirect
-	-	Untreated	DNP-BGG	4,308±753	28,560±4,115
-	-	Anti-Theta+C'	DNP-BGG	564±244	4,086±1,905
-	-	Anti-Theta+C'	None	85± 45	69± 23
BGG-primed	None	-	DNP-BGG	5,290±196	3,280± 453
BGG-primed	Anti-IgM	-	DNP-BGG	610±191	2,320± 941
BGG-primed	Anti-IgM	Anti-Theta+C'	DNP-BGG	1,693±774	37,187±3,772
Unprimed	Anti-IgM	Anti-Theta+C'	DNP-BGG	244± 88	1,829± 511

[a]A total of 50 x 10^6 cells (based on cell count before treatment) was transferred to lethally irradiated recipients along with the challenge antigen (100 µg) in a volume of 1.0 ml. In combinations, 25 x 10^6 cells from each population were transferred.

[b]Plaque-forming cells were assayed on day 6. Values represent mean standard error of 3 or 4 recipients per group after subtraction of nonspecific background.

[c]Anti-immunoglobulin serum (1/100) or anti-theta serum (1/50) were added to cell suspensions for 30 minutes at 0°C. Then complement was added at a 1/10 dilution for 30 minutes at 37°C. Cells were washed once and resuspended in original volume.

Also in Table III (p. 28) (compare line 4 with line 5) the effect of anti-IgM treatment on the carrier-primed spleen cells can be seen. At a 1/100 dilution, the direct PFC response was reduced almost 90%. However, the indirect response was reduced only 30%. This may be due to a lesser sensitivity of memory cells (IgG producers) to the anti-IgM serum, since their receptors may be of the IgG class (82-85). In later experiments, therefore, the antiserum was used at a 1/50 dilution, which was not of significantly higher toxicity toward thymocyte controls. In spite of the lack of complete effectiveness by antiserum treatment the contribution of this population to the combined populations' response (compare line 5 to line 6) is less than 3% when one considers comparable cell numbers. One other point to be drawn is that carrier-priming does, indeed, significantly increase the ability of the spleen cells to mount a response to the DNP hapten. In this case the increase is about 20-fold (compare line 7 to line 6).

The peak time for assay of DNP responses was determined by combining B and T cell populations. Thus, 25×10^6 "young" hapten-primed spleen cells that had been treated with anti-theta serum and complement (the stock B cell indicator population) were combined with 25×10^6 young or old carrier-primed spleen cells which had been treated with anti-IgM serum and complement (the test T cell helper populations) and injected into lethally irradiated mice along with a challenge dose of hapten-carrier conjugate (DNP-BGG, 100 µgm). DNP-specific plaque forming cells were assayed on days five through eight and the splenic indirect plaque forming cell responses are plotted in Figure 5. Note that peak B cell

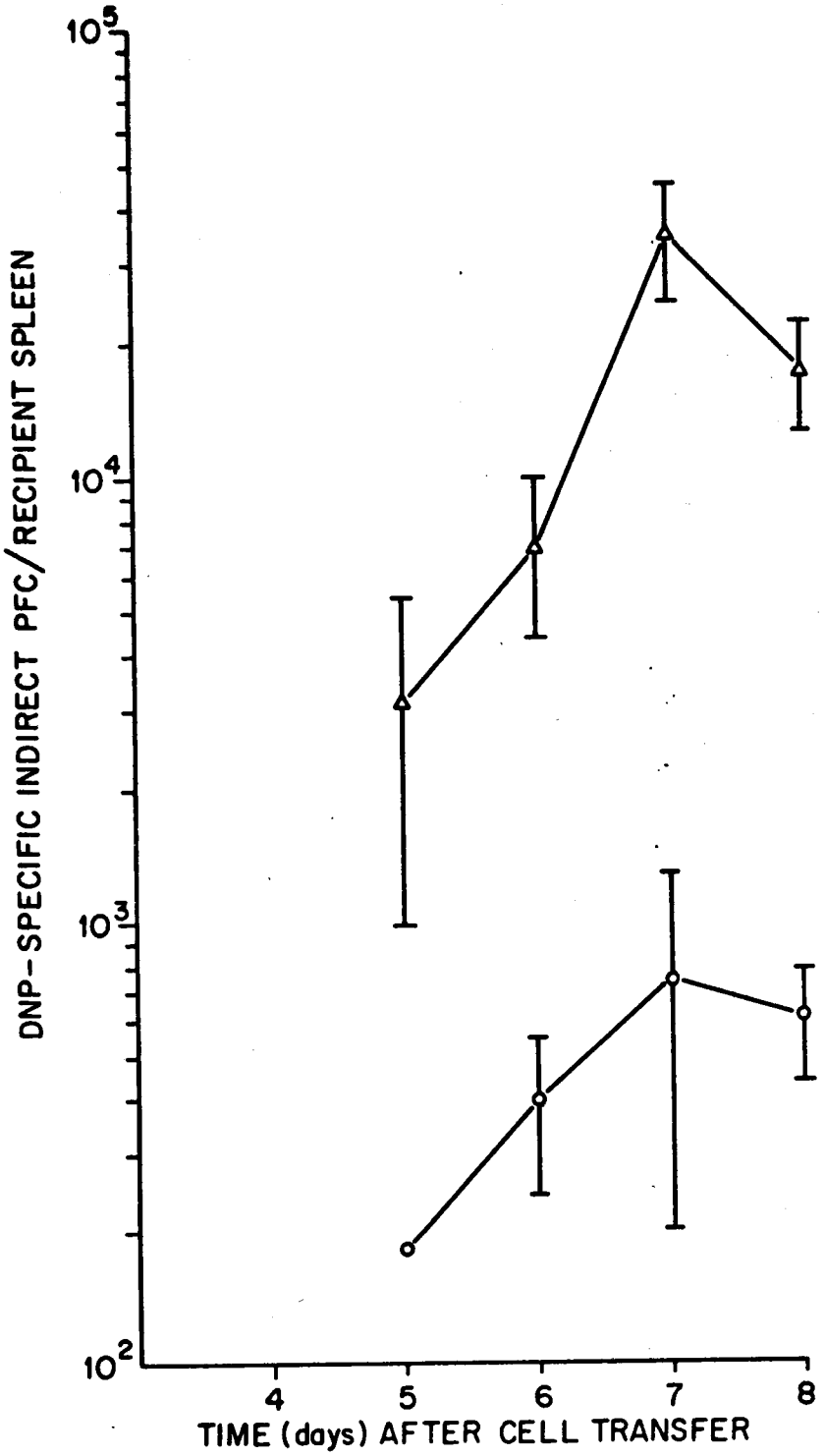

Figure 5. Kinetics of cell transfer system.

Anti-theta treated spleen cells (25×10^6) from $DNP_{14}HGG$ primed young mice and anti-IgM treated spleen cells (25×10^6) from BGG primed young (Δ) or old (O) mice were mixed with 100 micrograms of $DNP_{19}BGG$ on bentonite. Recipients (four per group) were irradiated with 800 R prior to transfer. Data for indirect plaques is presented. Vertical bars represent 95% confidence limits.

responses in the presence of either "young" or "old" T cells occur on day seven. The 50-fold difference in PFC per recipient spleen indicates a gross deficiency in the old mice. However, it should be noted that in later experiments the responses of both age groups were much higher and that this difference was not as exaggerated. This may be due to the fact that T cell donors were primed six weeks before this experiment rather than two weeks as in the later experiments. This kinetic aspect of T cell priming should be further explored.

Age-related changes in T cell helper function. Having established the experimental conditions necessary to demonstrate age-related differences in T cell helper function, a series of experiments was set up with mice of different age groups that were all primed with the same carrier-bentonite preparation (BGG) for identical time periods prior to assay (two weeks). All age groups were tested using B cells from a single group of young mice which were primed four weeks earlier with DNP-HGG on bentonite.

Figures 6 through 9 summarize the results of these experiments. Two or three spleens were pooled from each of the 4, 14, 19 and 34 month old age groups to compare the helper activity of the T cell populations. The dose-response curves for indirect plaque forming cells (solid lines), the major cell type responding (greater than 90% of the total response), show remarkable similarity. All have "break" points within the range of T cell doses tested and all continue to exhibit a rise in PFC after reaching the apparent "plateau" level.

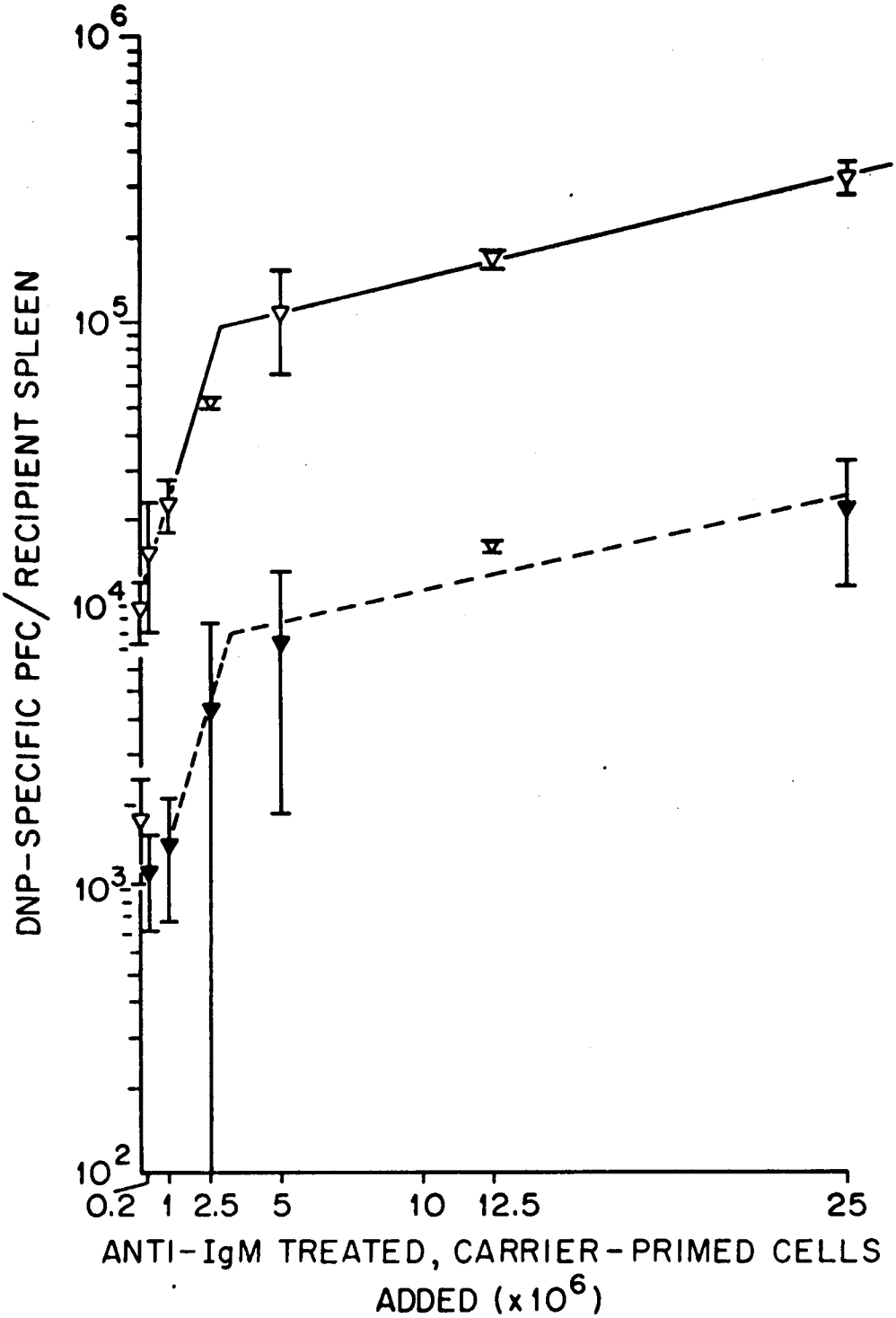

Figure 6. Response of DNP-primed "B" cells to DNP-BGG in the presence of graded numbers of BGG-primed "T" cells from 4 month old mice.

Dilutions of anti-IgM treated BGG-primed spleen cells from 4 month old mice were mixed with 25 x 10^6 anti-theta treated spleen cells from young DNP-primed mice and challenged with 100 micrograms of $DNP_{19}BGG$. Recipients, (3-4 per group) were irradiated with 800 R prior to transfer. DNP-specific PFC were assessed at 7 days. Solid lines represent indirect (75) PFC. Dashed lines represent direct (195) PFC. Vertical bars represent 95% confidence limits. Lines were fit by least squares regression analysis.

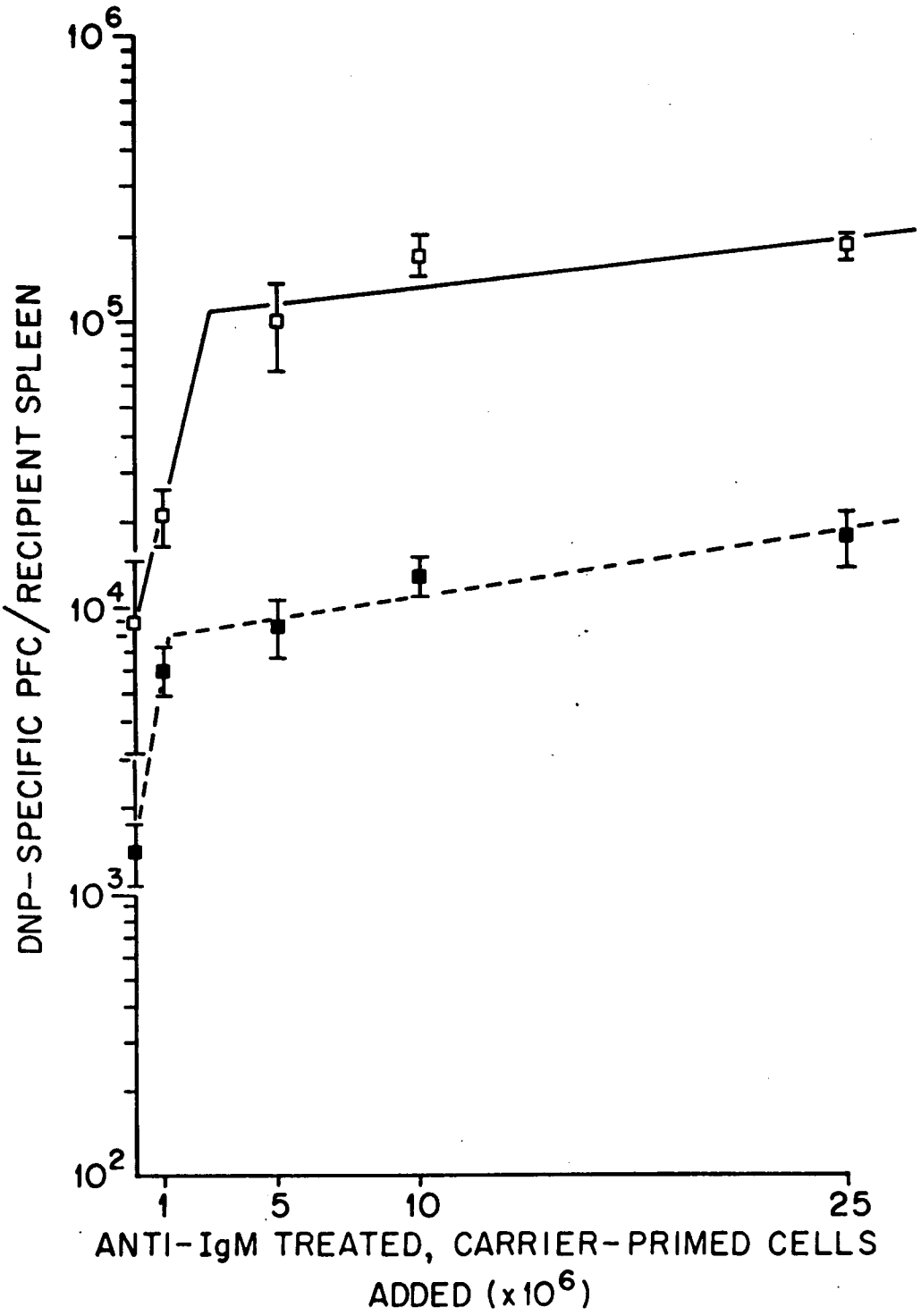

Figure 7. Response of DNP-primed "B" cells to DNP-BGG in the presence of graded numbers of BGG-primed cells were from 14 month old mice.

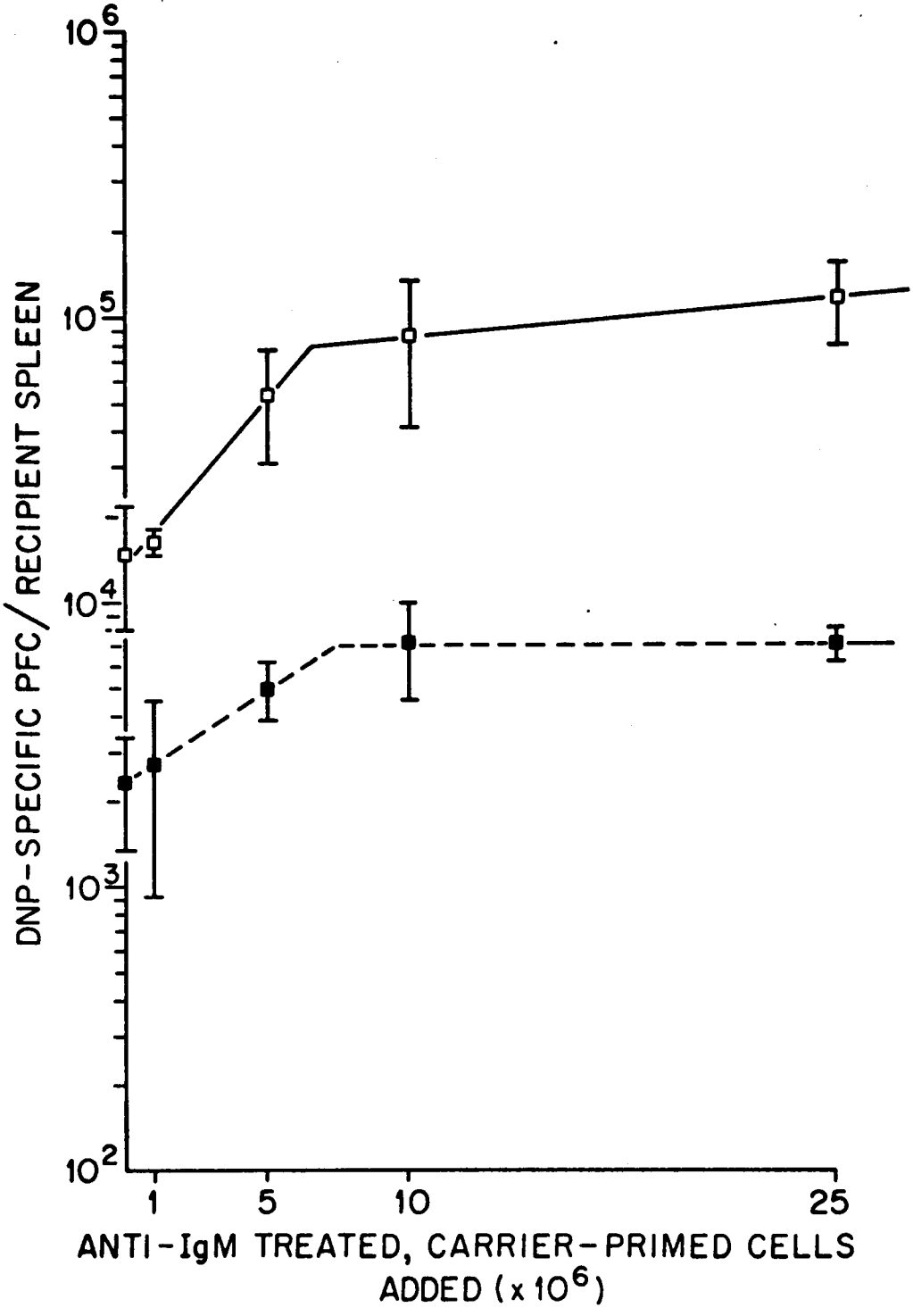

Figure 8. Response of DNP-primed "B" cells to DNP-BGG in the presence of graded numbers of BGG-primed cells were from 19 month old mice.

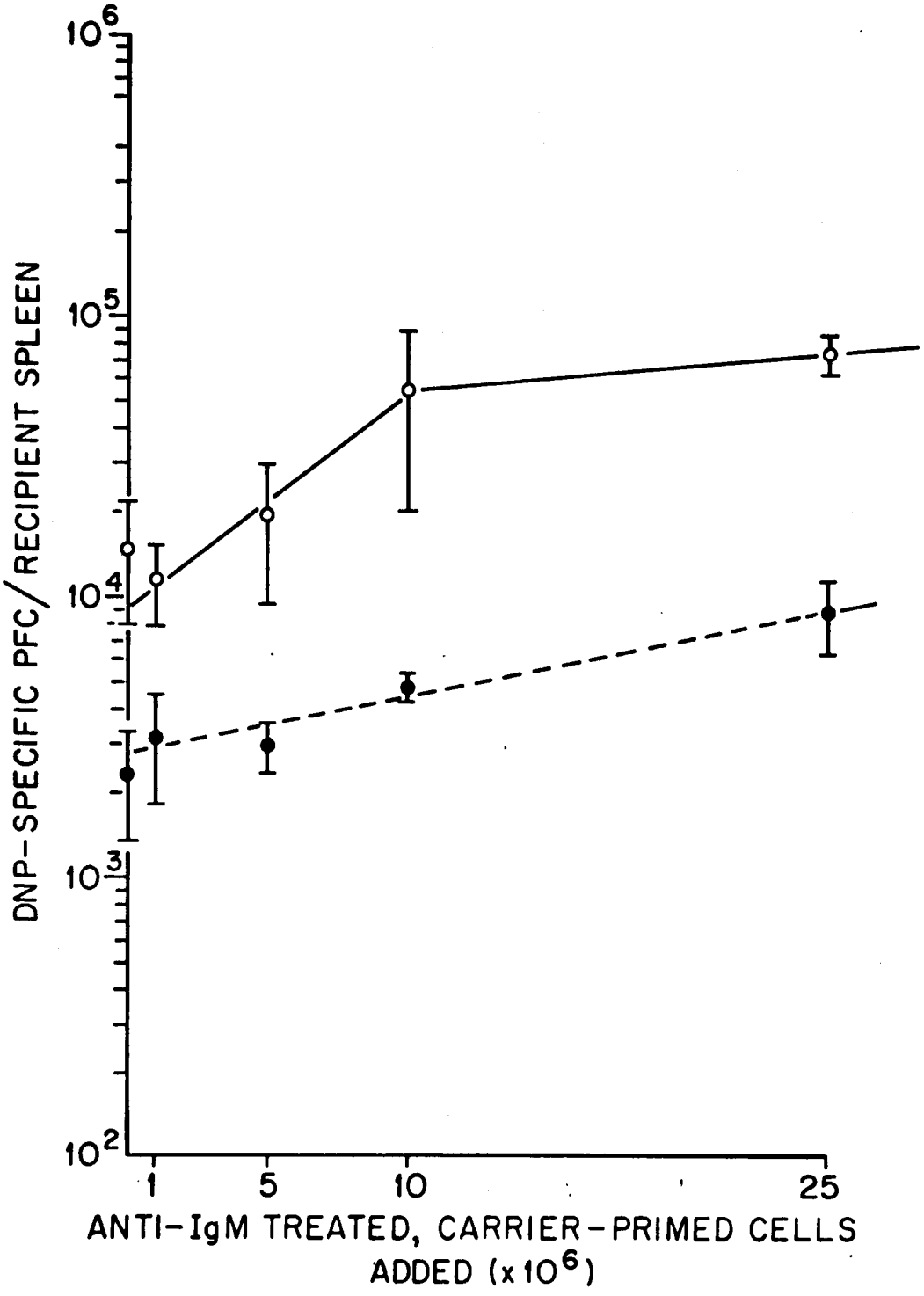

Figure 9. Response of DNP-primed "B" cells to DNP-BGG in the presence of graded numbers of BGG-primed cells were from 34 month old mice.

The "break" point in this type of dose-response analysis represents the point at which T cells become limiting (86), that is, at lower doses of T cells there are DNP-sensitive B cells which do not differentiate or proliferate for lack of T cell help. In this steep portion of the curve, then, B cells are in excess or, in other words, the limiting numbers of T cells are functioning at full capacity. The break points for the indirect PFC (7S) of the four age groups are plotted in Figure 10 as a function of age. The steady increase in T cell dose at the break point observed after 14 months of age is indicative of decreasing helper function.

The rate of increase (slope) in the indirect plaque forming cells after reaching the "plateau" is significantly ($P<.05$) greater in the young adult (4 month old) mice than in the older age groups. The significance of this slope with respect to T cell regulation is discussed below.

For direct plaque forming cells (Fig. 6-9, broken line, p. 32-35), the shape of the dose-response curves is similar to the indirect curves except that for the 19 month old curve which has no rise after reaching the break point. Furthermore, the 34 month old curve showed no detectable break within the dose range tested. Since the indirect plaque forming cell response, which has been shown to be more highly T cell dependent (see 87 for review), accounted for more than 90% of the total response in all cases, we have focused our attention on this component of the immune response as being more representative of T cell helper function.

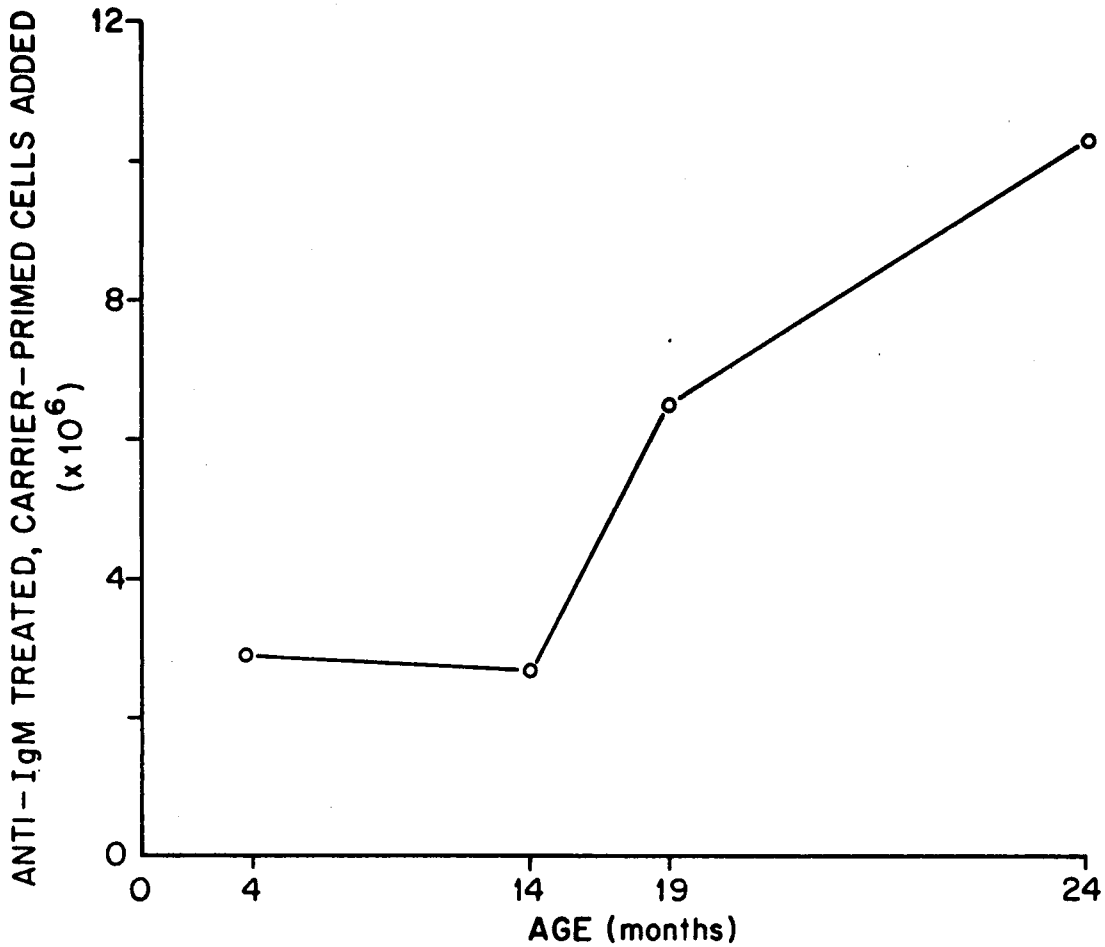

Figure 10. Break points of dose-response curves (Figs. 6-9) as a function of age of the "T" cell donor.

Both the indirect and direct plaque forming cell responses obtained at the maximum dose of T cells tested (25 $x10^6$) are plotted in Figure 11 as a function of age of the T cell population. The data include an additional point obtained from a single 31 month old mouse spleen. The difference in helper function as measured in this system is 4- to 5-fold over the age span studied. The regression line indicates a 3.5-fold decrease in helper function at the mean of the life span for this strain (30 months) when compared to fully competent young adults (4 months old).

Discussion

In the data presented here, the capacity of carrier-primed old T cells to cooperate with hapten-primed young B cells in response to the hapten-carrier conjugate, DNP-BGG, was shown to be deficient. Thirty-four month old splenic T cells provide only one-fourth of the helper capacity found in identically primed four month old splenic T cells.

Before conclusions can be drawn concerning specific T cell functional deficiencies, one basic parameter must be established, i.e., the relative frequencies of antigen-reactive T cells in the spleen of old and young carrier primed animals. Experimentally, this could be determined by limiting dilution analysis, wherein the frequency of helper cells would be extrapolated from the plotted percentages of nonresponding animals receiving decreasing dilutions of T cells simultaneously with a constant number of B cells. Unfortunately, the

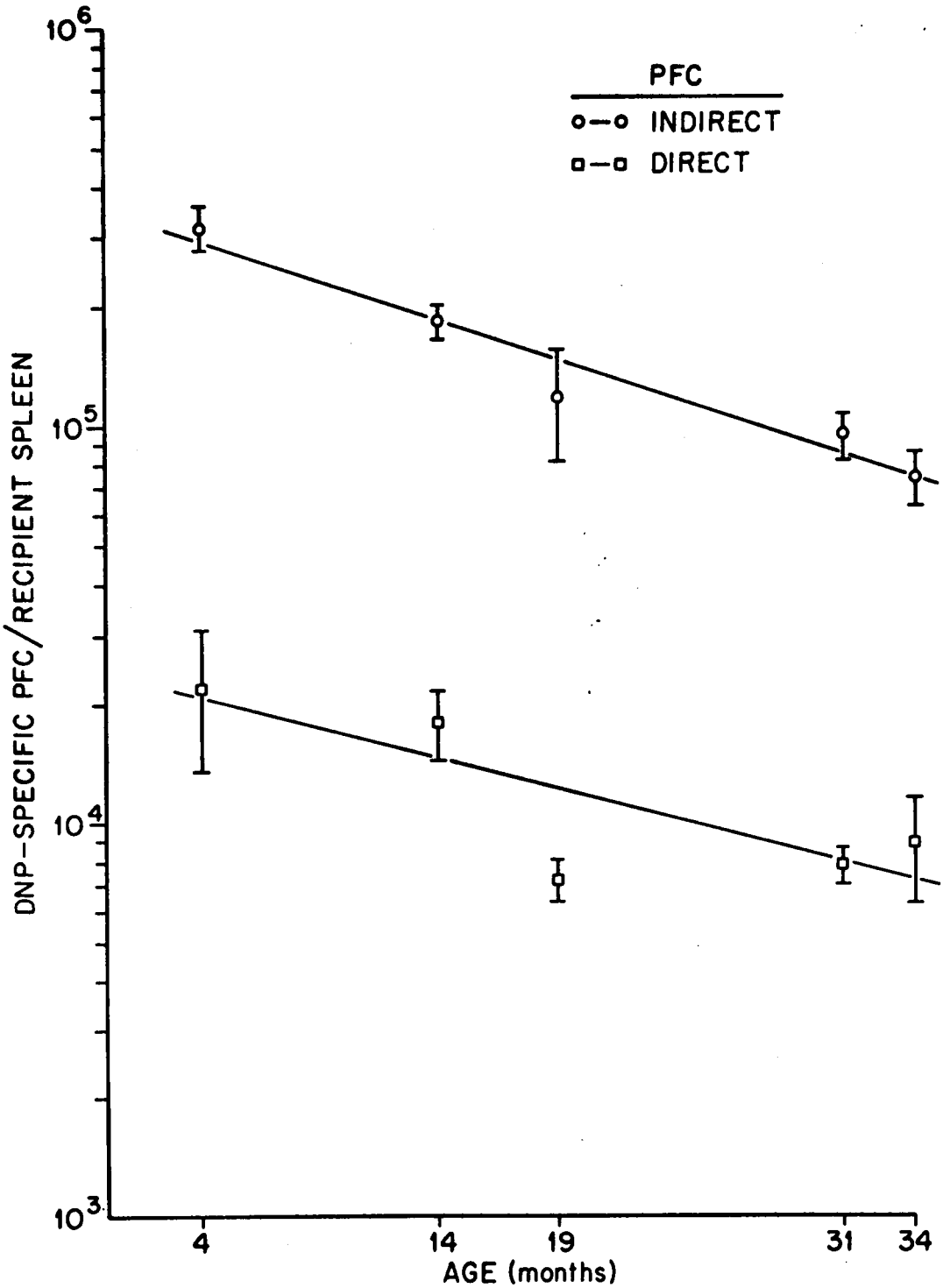

Figure 11. Helper effect of 25 x 10^6 carrier-primed cells as a function of age.

Vertical bars indicate 95% confidence limits. Lines were fit by least squares regression analysis.

number of animals required for such investigations was far greater than the number available. Therefore the data will be interpreted with respect to the possibilities that the frequency of helper cells in old animals is (a) equal to, (b) greater than, or (c) less than that of young mice.

Assuming that the frequency of helper cells in the old mice is the same as in the young, we can gain insight into the relative activity of the T cells by considering the model of Groves et al., (86). According to this model, the interaction of two cell types is necessary for the development of antibody forming cells (AFC): the antigen reactive T cell (ART) and the precursor to the AFC which is the DNP-sensitive B cell in this case. A dose-response assay (such as those presented in the results section) can be described by the equation:

$$R = \Phi X \left\{\frac{p(X)S}{1 - [1 - p(X)]}\right\} 2^y$$

where R = PFC/spleen

Φ = frequency of antigen-reactive T cells (ART)

X = cell dose (anti-IgM treated carrier-primed cells)

p(X) = probability that a "site" on the ART will react with a DNP-sensitive B cell

S = number of "sites" per ART, and

y = number of divisions which the B cell undergoes after stimulation.

The "sites" on the ART need not be limited to sites of physical contact, but, in a broader sense, can be interpreted as the capacity of the T cell to provide soluble factors (88-94) for interaction with the B cell

if these are considered to be short range effectors (Groves, D. L., personal communication and 90, 34) or to act via macrophages (95-98).

At the dose of T cells where p(X) becomes equal to 1.0, there is an inflection, or "break" point. Thus, for lower doses of T cells (equivalent to higher relative doses of B cells), the probability for interaction of each of the T cell sites is unity. At the break point, we can compare the relative effectiveness of the young and old carrier-primed populations. Thus, when p(X) = 1.0, the equation reduces to:

$$R = \Phi X\{S\}2^{Y}.$$

Since the precursor cell population in this comparison is uniform, the last term cancels out when we make a ratio of the old to young PFC responses.

$$\frac{R_{Old}}{R_{Young}} = \frac{\Phi_O X_O S_O}{\Phi_Y X_Y S_Y}.$$

Assuming, then, that $\Phi_O = \Phi_Y$, for 34 month old versus 4 month old mice, the data in Figures 6 and 9 (p. 32, 35) can be expressed as follows:

$$\frac{R_O}{R_Y} = \frac{X_O S_O}{X_Y S_Y} = \frac{10.3 \times 10^6 \; S_O}{3.0 \times 10^6 \; S_Y} = \frac{56 \times 10^3 \text{ PFC}}{100 \times 10^3 \text{ PFC}}.$$

Where, at a dose of 10.3×10^6 old T cells the response was 56×10^3 PFC and at 3×10^6 young T cells the response was 100×10^3 PFC. Therefore, the ratio $S_O/S_Y = 0.16$, which means that old cells have about one sixth as many "sites" (capacity) for interacting with B cells.

Assuming a 2-fold increase in the relative frequency of T cells in the spleen of old animals (54), the $\Phi_O = 2\Phi_Y$ for 34 month old versus 4 month old mice and $S_O/S_Y = 0.08$, implying an even greater deficiency

of sites in the old animals. If, on the other hand, there are half as many old T cells ($\Phi_O = 1/2\Phi_Y$), then $S_O/S_Y = 0.32$, or about one third of the young helper T cell capacity. One can see that in order to assume equal functional capacity, old mice must have only one sixth as many helper cells as young mice.

Which of the relative frequencies seems most reasonable in light of the presently available literature? When considering the results of Price and Makinodan (54), who found the frequency of unprimed old T cells is twice that found in young, the second assumption ($\Phi_O = 2\Phi_Y$) may be correct. This probably is an upper limit of the relative frequency of T cells since the secondary type response used in the present experiments could require proliferation of the T cells (61), which is known to be defective in old animals (56, 57). Thus the relative frequency of old to young T cells would be lessened.

On the other hand, Kruger and Gershon (99) have recently introduced evidence that a majority (40-70%) of the memory T cells in a secondary response are not the progeny of antigen induced mitoses but may be "recruited" by the long-lived memory cells from naive T cells, or become memory cells without dividing. If this is the case, then the 2-fold frequency advantage of the old spleen cells (54) would be maintained only for the nondividing part of the memory population while the mitotically derived population would be deficient relative to the young. (Using a T cell specific mitogen, PHA, Hung et al., (57) have shown that 4-fold fewer labelled blast cells result from division in old spleen cell suspensions.) Thus, for example, if there were 100

memory cells in the young spleen, we would expect 40 to 70 nondivided and 60 to 30 progeny from mitosis. In comparison, the old spleen would contain 80 to 140 nondivided cells and 15 to 8 progeny from mitosis. Therefore the total old memory T cell compartment would be 95 to 148 percent of the young T cell memory compartment. These calculations put the frequency in the range of our first assumption, that the frequency of old T cells is approximately equal to the young T cells.

The experiments of Kruger and Gershon may be in error, however, since the interval between injections of ^{3}H-thymidine (six hours) used in their experiments is comparable to the total S phase of dividing lymphocytes (five to seven hours) (63). These authors set their minimum cutoff for positively labelled cells at ten autoradiographic grains, which represents 5 x 10^{6} molecules of thymidine per cell in their system. Using these criteria under their conditions, they possibly overlooked a large number of divided cells. This error could be considerable because Gelfant and Perkins (personal communication) have found by increasing the frequency of injection of ^{3}H-thymidine from once every four hours to once every two hours that the number of labelled plaque forming cells is more than doubled in their study of precursors of the primary response. In relation to our discussion of old versus young memory cell compartments, then, more memory T cells arising through mitotic response to antigen would decrease the ratio of frequencies to a minimum of one half ($\Phi_0 = 1/2\Phi_Y$). This minimum represents the case where <u>all</u> memory cells result from mitosis and where we assume a 4-fold

difference in the progeny of a given set of mitoses, as suggested by the data of Hung et al. (57).

The rise in the plateau region of the dose-response data is similar to the "premium effect" described by Celada (100), wherein an increasing response on a per cell basis is seen with increasing numbers of spleen cells transferred to irradiated recipients. If the present system is "saturated" with T cells at the "break" point, as postulated by the Groves model, then no increase in PFC should be seen and the line should be parallel to the abscissa. That this is not the case suggests that further regulatory functions are being exerted by the T cells.

The continued rise in PFC cannot be attributed to B cells in the carrier-primed population since control experiments (Table III, line 5 and 6, p. 28) show that only 3% of the response generated by mixing carrier-primed and hapten-primed cells is contributed by the carrier-primed cells alone.

Janeway (78) has described a similar situation in his investigations of the DNP response and suggests that the "premium" effect is due to the interaction of two types of T cells (both carrier-primed) or two functions of the same type T cell. Thus, one function would be to activate the clones of DNP-sensitive B cells while the second would be to expand the clones through cellular proliferation (44). Increasing the number of T cells after the break point in the dose-response data (Figures 6-9, p. 32-35) might then cause increased clonal expansion, thus increasing the resultant number of plaque forming cells per spleen.

Comparing old and young "plateau" responses with respect to this latter point, a slight deficiency in older animals is apparent since the 4 month old slope is significantly greater than those of older age groups. Nevertheless, the proposed clonal expansion function is still noticeable even in T cells from 34 month old mice. Alternatively, the difference in these "plateau" slopes could be due, again, to differences in relative T cell frequencies.

The presence of suppressor T cells (see review, 86) cannot be ruled out in these results, in fact one might explain the differences in age animals on the basis of increased suppressive activity, however there is no obvious evidence in these experiments. On the other hand, it was of interest that in one similar experiment, done with 33 month old mice which had been primed 9 weeks earlier, a significant suppression of the response was seen when compared with hapten-primed cells alone. Possibly the length of time after carrier priming was a factor in this result.

In conclusion, the data present evidence of age-related deficiency in the helper function of murine splenic T cells. If we assume equal frequencies of antigen-reactive T cells in both young and old spleens, then the age-related deficiency appears to be on the order of 6-fold with respect to the ability to activate DNP-sensitive B cells and about 4-fold with respect to the ability to promote clonal proliferation. This dual function of the T cells apparent in the data presented here is in accord with the two-signal theories of B cell activation (see

reviews in 22) if one considers that the first signal causes proliferation of sensitive B cells and the second signal causes differentiation of the activated cells into antibody secreting cells.

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