STUDIES OF THE REGULATORY EFFECTS OF THE SEX HORMONES ON ANTIBODY FORMATION AND STEM CELL DIFFERENTIATION*

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The capacity of the female of various mammalian species, including man, to outperform the male when measured in terms of immune responsiveness has been documented many times. Depending on such variables as age of host, presence of associated disease, or experimental conditions, enhanced antibody and/or serum levels of IgA (1, 2), IgM (2–4), and IgG (5) have been observed in females in comparison with levels in control groups of males. The capacity for enhanced antibody formation is manifested both in secondary as well as in primary immune responses (4, 6).

The enhanced immunological capability, as perhaps might have been predicted, has been associated with numerous additional observations and consequences. For example, it is apparently easier to produce immunological tolerance in the male than in the female (7). The female rodent experiences enhanced susceptibility to autoimmune disease produced by circulating antibody (8, 9) as well as by immune complexes (10). A similar increased incidence in the human female has been documented frequently.

It has also been recorded that the capacity for cell-mediated immunity is greater in females than in males. Thus, females reject tumors and homografts with greater efficiency than males (4, 11–13), and cells derived from females perform better in mixed lymphocyte cultures than cells derived from males (14).

Despite the abundant evidence which has accumulated for enhanced immunological response in the female, the underlying mechanisms to account for these differences have not been adequately defined. The possibility that lymphoid tissue is involved in these variations is suggested by several observations. It is well documented that both androgens and estrogens produce lymphoid atrophy of both thymus and peripheral lymphoid organs (15–18). Conversely, gonadectomy both in the male (19) and in the female (5, 19) has been associated with lymphoid hyperplasia. Adrenalectomy also enhances antibody production (20) presumably by generating lymphoid hyperplasia

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(16), arising as a consequence of excision of additional androgen or adrenocorticosteroid-producing tissue.

In the present work a beginning has been made in defining the mechanisms underlying the marked effect of sex hormones on immune responsiveness. In order to evaluate the potential role of the thymus-derived and bone marrow-derived lymphocytes, comparison of the immune response in normal males and females to highly thymus-dependent and thymus-independent antigens was undertaken. For comparison with these two groups, evaluation was also made of the immune response in gonadectomized males, a procedure which others have shown to convert the male to a female in terms of immunological responsiveness (5, 12). As an indirect assessment of the contribution of lymphoid tissue, thymus and spleen weights were determined in the three groups of mice and correlated with changes in numbers of circulating lymphocytes. Additional studies pertaining to the role of the thymus gland in generating the enhanced responses in gonadectomized males were performed by evaluating immune responsiveness in groups of mice which had been simultaneously thymectomized and gonadectomized. The percentage of cortisone-resistant thymus cells was also evaluated in the three experimental groups of mice. Finally, a possible role of altered stem cell differentiation was considered indirectly by assessing the iron-59 uptake of lethally irradiated gonadectomized and normal males, and females reconstituted with bone marrow cells.

Materials and Methods

Animals.—Mice used in the following experiments were mainly of the CAF_1 and $B6D2F_1$ inbred strains (purchased from Jackson Laboratory, Bar Harbor, Maine), although an outbred Swiss mouse strain (Taconic Animal Farms, Germantown, N. Y.) was also employed in some experiments. Experimental and control groups consisted of 6–12 mice. Animals were 5-6 wk of age at the commencement of the experiments. They were fed on a Rockland Teklad (Teklad, Inc., Monmouth, Ill.) diet of mouse pellets and water, *ad libitum*.

Antigens.—Various antigens were used in the experiments. After initial trials with a variety, most experiments were carried out with horse and goose erythrocytes as examples of thymus-dependent antigens (R. Kerbel, unpublished observations; A. J. S. Davies, unpublished observations) and polyvinylpyrrolidone (PVP)[1] as a thymus-independent antigen (21, 22). Antigens were generally given intravenously in a dosage which will be indicated in each experiment.

Assays of hemagglutinins were carried out employing microtitrator equipment (Fisher Scientific Co. Canada Ltd., Toronto, Ontario). A relative measure of 19S and 7S hemagglutinins was performed employing differential sensitivity with 2-mercaptoethanol (23). Assays of antibodies to PVP were carried out employing suitably tanned erythrocytes as previously described (21).

Operative Techniques.—The testes of 6-wk old male mice were removed under ether anesthesia via a small midline incision of the scrotal skin. Mice aged 5 wk were thymectomized employing suction for removal of the thymus. Thus, in the experimental groups in which combined thymectomy-orchiectomy was performed, removal of the gonads was carried out in mice surviving thymectomy at an interval of 1 wk.

[1] *Abbreviation used in this paper:* PVP, polyvinylpyrrolidone.

Determination of Spleen and Thymus Weights.—The thymuses and spleens of the various mice were dissected free of fibrous connective tissue and weighed on a sensitive Mettler balance (Mettler Instrument Corp., Princeton, N. J.) The weights were recorded to the nearest milligram. In some experiments the body weights of the mice were determined before excision of the organs.

Determination of Nucleated Organ and Peripheral Cell Counts.—For the determination of numbers of nucleated cells per spleen or thymus, individual lymphoid organs were pressed through an 80 mesh stainless steel grid into a Petri dish containing either 2 or 5 cc of medium (depending on the individual experiment). Nucleated cell counts were performed routinely with a hemacytometer. Counts were expressed as cells per cubic centimeter or per thymus or spleen. For the determination of the number of cortisone-resistant cells, mice were given an injection of cortisone acetate (kindly provided by Dr. Edward L. Masson, Upjohn Company of Canada, Don Mills, Ontario) in a dosage of 125 mg/kg body weight. 48 hr later the thymus glands were removed and cell suspensions prepared as described. This period corresponds to the time of onset of maximum reduction of thymic cell number after this dosage of cortisone acetate (24).

Irradiation.—In the single experiment in which irradiation was employed, mice housed in a Lucite chamber were lethally irradiated with 850 R, employing a Gammacell 20 irradiator (Atomic Energy of Canada Ltd., Ottawa, Ontario). The irradiation source was cesium-137 administered in a dosage of 117 rads/minute.

Determination of Iron-59 Uptake.—Lethally irradiated mice were reconstituted with either 10^5, 10^6, or 10^7 bone marrow cells derived from syngeneic recipients. During the late afternoon of the 6th day a tracer dose of 0.2 μCi of iron-59 was administered. The following morning the mice were sacrificed, individual spleens removed, the tissue solubilized with tissue solubilizer (Amersham/Searle Corp., Toronto, Ontario), and the gamma emission determined in a Packard gamma counter (Packard Instrument Co., Downers Grove, Ill.). The data were expressed as counts per spleen.

Determination of Spleen Colonies.—Lethally irradiated groups of mice were injected with 10^5 bone marrow cells. Either 8 or 9 days later the mice were sacrificed, and individual spleens excised and deposited in small vials containing Bouin's fixative. After 2 or more hr the number of spleen colonies was counted.

Statistical Methods.—The titers, cell counts, colony counts, and uptake of radioactivity were compared employing a Student's t test. The data were considered significant when the P values were less than 0.05.

RESULTS

Comparison of the Primary and Secondary Immune Responses to Thymus-Dependent and Independent Antigens.—Initial experiments demonstrated that the enhanced capacity for humoral antibody formation in gonadectomized male mice did not occur with regularity until 10 wk had elapsed postorchiectomy. Since orchiectomy was regularly performed at 6 wk of age, the comparative experiments were undertaken in mice 16 wk of age or older.

Fig. 1 illustrates the comparative responses of normal male and female mice and orchiectomized male mice immunized intravenously with 0.1 μg of PVP (360,000 mol wt). It may be seen that the immune response of females exceeded that of the male mice, while the castrated mice generated levels significantly higher than those of female mice. In some additional experiments employing this thymus-independent antigen, levels in castrates were equivalent

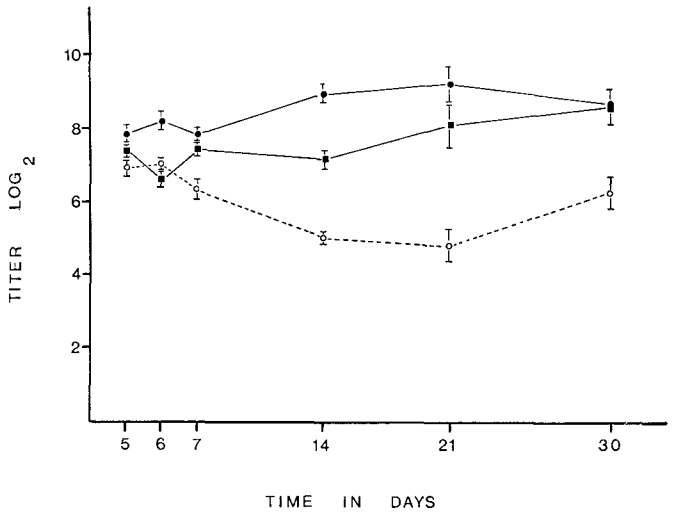

FIG. 1. Primary immune response of normal females and normal and gonadectomized males immunized intravenously with 0.1 μg of PVP at 16 wk of age, 10 wk after orchiectomy. Points indicate mean and SE of 8–10 B6D2F$_1$ mice per group. Castrated males, (●—●); normal males, (○-------○); normal females, (■—■).

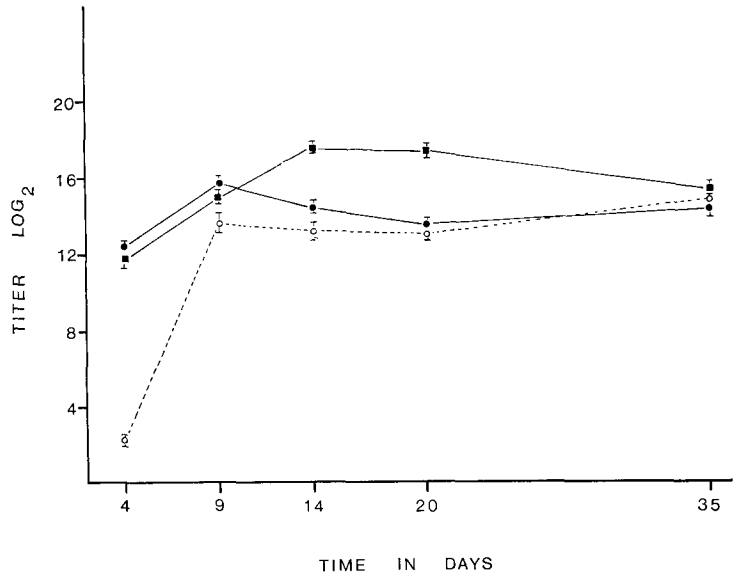

FIG. 2. Primary immune response of males, females, and orchiectomized males after intravenous administration of 0.2 cc of 10% horse erythrocytes. Animals were 16 wk of age, 10 wk after gonadectomy. Each point represents mean and SE of 9–12 B6D2F$_1$ mice per group. Gonadectomized males, (●—●); normal males, (○-------○); normal females, (■—■).

to those in females. For the determination of the differential immune response to thymus-dependent antigens, numerous experiments were carried out employing groups of normal male and female mice and orchiectomized mice of equivalent age, immunized with either 0.2 cc of 10% horse erythrocytes, rat erythrocytes, or goose erythrocytes intravenously. Fig. 2 illustrates the data derived from a typical experiment employing horse erythrocytes. The female mice responded better than the normal male mice throughout both the early 19S phase as well as the subsequent 7S phase of the response. The early 19S phase of the response of castrated mice was characterized by levels of 2-mercaptoethanol-sensitive hemagglutinating antibody in excess of those reached in the female mice. Subsequently, the levels of 2-mercaptoethanol-resistant hemagglutinins were com-

TABLE I

Summary of Experience in Evaluation of Enhancement of Antibody Formation in Gonadectomized Male Mice*

Antigen employed‡	No. of experiments	No. of positives	No. of negatives
PVP	7	6	1
Goose RBC	33	17	16
Rat RBC	21	4	17
Horse RBC	17	9	8

* Numbers of positives denotes those experiments in which statistical significance was established. In practice, this corresponded to observation of at least a 2 $\log_2$ difference throughout the time-course of the experiments. Negatives were not evaluated statistically. No experiments were seen in which responses of normal male exceeded those of castrated animals.

‡ The response to PVP, the thymus-independent antigen, was always evaluated in mice immunized intravenously with 0.1 μg, which was shown to be the optimal dose for immunization. In the other experiments with thymus-dependent erythrocyte antigens, various dosage schedules were employed, although 0.2 cc of 10% erythrocytes intravenously was the usual dose.

parable with the titers observed in normal males, and significantly lower than levels in female mice. The more rapid appearance of circulating antibody in females and castrates was also regularly observed.

Numerous additional experiments were then carried out omitting the use of female mice for the purpose of economy, in order to determine the reproducibility of the changes in response of gonadectomized male mice. In general, the development of enhanced levels of antibody during the 19S phase of the immune response to thymus-dependent antigens together with a more rapid appearance of circulating 19S antibody was confirmed in the castrated mice when compared with normal male mice. However, comparisons of the 7S phase indicated the primary responses of males and gonadectomized males to be extremely variable. Table I summarizes our total experience. In some experiments 7S antibody levels in castrates were higher than in normal males. In many other instances differences could not be demonstrated. Although the reasons for

these variations remain obscure, failure to demonstrate enhancement was believed in part to be due to seasonal variation; the enhancement in 7S levels between the mice was more regularly produced during the summer months than in the winter months. There was some indication also that strain differences represented a major factor. For example, the BALB/c mice in three experiments

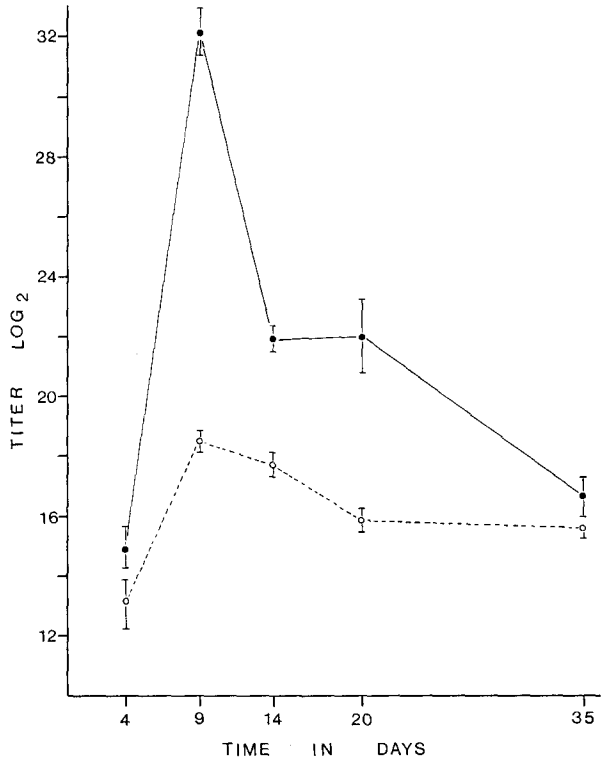

FIG. 3. Tertiary immune response of gonadectomized and normal males immunized intravenously with 0.1 cc of 1% rat erythrocytes at 16 wk of age, 10 wk postorchiectomy. These animals had been primed by two injections of 0.1 cc of 10% rat erythrocytes at 2 and 4 wk of age. Points indicate mean and SE of eight Swiss white mice per group. Castrated males, (●—●); normal males, (○-------○).

regularly yielded very significant enhancement in orchiectomized mice, but their cost and lack of availability in bulk numbers precluded their use. In addition, some of the differences may have been due to differential content of estrogen in the grain contained in the mouse diets, as reported by the manufacturer.

Experiments to evaluate levels of secondary antibody production were performed in normal males and females as well as in castrated males. In these experiments a second injection of 0.1 cc of 1% erythrocyte antigen was given at

about 30–40 days after the initial immunization. In most cases the levels of antibody reached in the castrated male was equivalent to the levels reached in the female, and in both these groups the levels exceeded those attained in the normal male of equivalent age. In these instances the antibody was resistant to 2-mercaptoethanol, and therefore, presumed to be 7S antibody.

The influence of castration on secondary and tertiary immune responsiveness

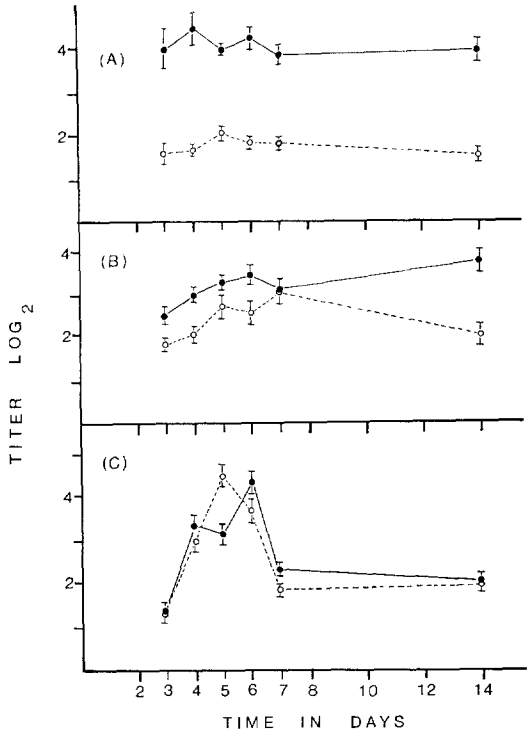

FIG. 4. Primary immune response of normal and castrated Swiss white male mice immunized intravenously with 0.1 μg of PVP, 10 wk (Fig. 4 A), 20 wk (Fig. 4 B), and 30 wk (Fig. 4 C) after gonadectomy at 6 wk of age. Each point indicates the mean and SE of 8–10 mice. Castrated males, (●—●); normal males, (○-------○).

was most dramatically represented in groups of mice immunized with either a second or third injection of 1% rat erythrocytes at 16 wk of age, 10 wk after castration, which was carried out after prior administration of either one or two doses of 0.1 cc of 10% rat erythrocytes. Fig. 3 illustrates the data from one experiment. It may be seen that orchiectomy profoundly influenced levels of antibody formation even when carried out subsequent to priming of the animal with that antigen.

The duration of the enhancement effect was studied in groups of Swiss white mice immunized intravenously with 0.1 μg of PVP, 10, 20, or 30 wk after cas-

tration at 6 wk of age. From the data presented in Fig. 4, it may be seen that the enhanced response to PVP was diminished when animals were challenged 30 wk after orchiectomy. A disappearance of the enhancement effect was noted when responses to thymus-dependent antigens were determined with increasing time after gonadectomy. Finally, gonadectomy of two groups of older male mice, aged 9 and 10 months, failed to yield significant enhancement of antibody production when tested 10 wk later.

Effects of Sex Hormones on Lymphoid Tissue.—The next experiments were carried out in order to determine the changes in lymphoid organ weight and cell

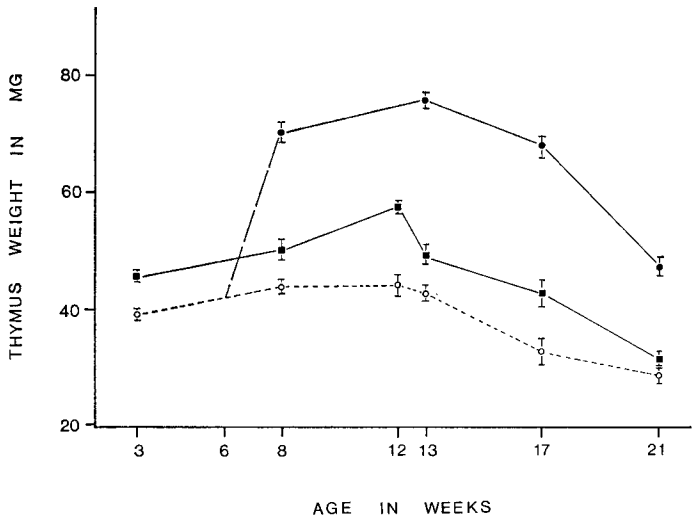

FIG. 5. Thymus weight of normal male and female B6D2F$_1$ mice and of males orchiectomized at 6 wk of age. Each point represents mean and SE of 12 mice per group. Gonadectomized males, (●—●); normal males, (○--------○); normal females, (■—■).

number after orchiectomy at 6 wk of age. These organ-extirpated mice were compared with normal male and female mice of equivalent age.

Fig. 5 indicates the relative thymus weight of the three groups of animals. In control male and female mice the increased thymus weight of females was already apparent even at 3 wk of age. After orchiectomy at 6 wk of age the thymus weight of operated mice increased dramatically. A significant increase in thymus weight was noted as early as 2 wk after orchiectomy. Throughout the period of observation the weight of the thymus of the castrated animal greatly exceeded the weight of the thymus from female animals, which in turn, exceeded the thymic weight in the male.

The body weights of the three groups of mice during the period from 8 to 16 wk of age varied, the males weighing 24–26 g, the females 20–22 g, and the gonadectomized males 22–24 g. Taking body weights into account and ex-

pressing the thymus weights recorded in Fig. 5 per unit body weight, the differences in thymus weight were even more striking.

Similarly, the spleen weight per body weight analyses revealed that the order of differences between the three groups of mice was the same as that recorded for the thymus. Spleens of castrated male mice weighed more than the organs of females, which in turn, exceeded the weights of spleens of normal male animals.

Fig. 6 illustrates the peripheral white counts obtained in the three groups of animals. It may be seen that the levels of peripheral white cells were greater in

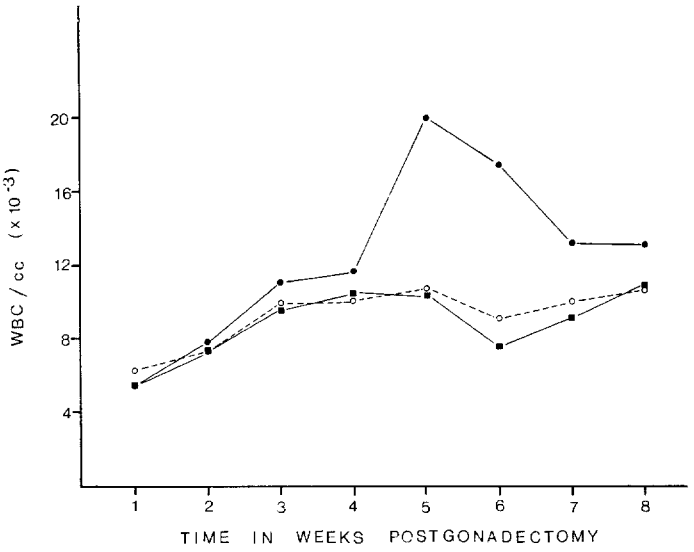

FIG. 6. Peripheral white blood cell counts of the three groups of B6D2F$_1$ mice. Each point represents mean of duplicate assays on a pooled blood sample derived from bleedings of 12 mice. Castrated males, (●—●); normal males, (○--------○); normal females, (■—■).

castrated males than in either of the other two groups, which were essentially equivalent in terms of total numbers of peripheral cells.

Various investigators have delineated the immunocompetence of the cortisone-resistant population contained in the thymus gland (24). Consequently, it was considered essential to document the numbers of cortisone-resistant cells in the thymus glands of castrated male mice and normal male and female mice. Figs. 7 and 8 illustrate the data comparing the numbers of thymus and spleen cells, respectively, before and 48 hr subsequent to administration of cortisone acetate in a dose of 125 mg/kg body weight. It may be seen from the results derived from assays 4, 7, and 10 wk after castration that the absolute numbers of cortisone-resistant thymus cells were equivalent in all three groups both in spleen and in thymus. Thus, the increase in lymphoid organ weight resides in the increased size of the cortisone-sensitive lymphoid population.

Role of the Thymus in Enhancement of Antibody Formation in Castrated Mice.— The marked hyperplasia of the thymus gland after orchiectomy at 6 wk of age raised the question as to whether the enhancement of immune response in castrated mice represented a hyperfunction of this tissue. In order to investigate its role, mice were thymectomized at the age of 5 wk and sets of surviving male mice were castrated 1 wk later. At the age of 16 wk the immune response of thymectomized, castrated mice to PVP was evaluated. The response of these mice was compared with male mice sham-thymectomized and castrated, as well as to thymectomized and normal male and female mice.

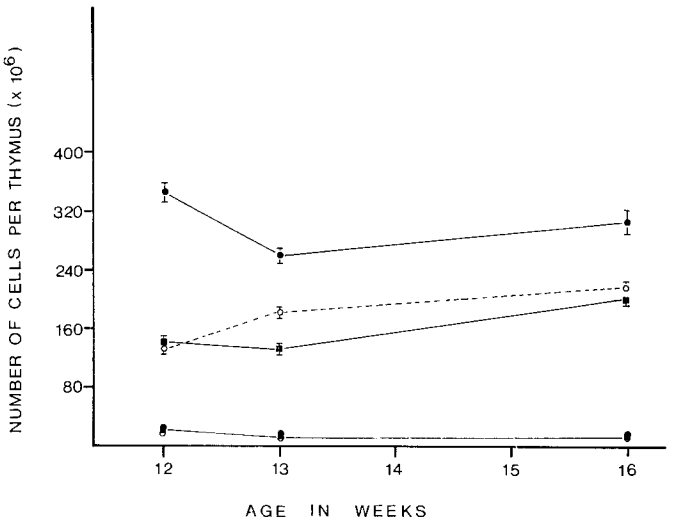

FIG. 7. Total nucleated cell counts of thymuses of normal and castrated male and female B6D2F1 mice of identical age, before and 48 hr after injection of cortisone acetate intraperitoneally in a dose of 125 mg/kg body weight. After cortisone administration, the number of recoverable cells was virtually identical; hence, the data are expressed as a single line for clarity. Each point represents the mean and SE of 12 mice per group. Castrated males, (●—●); normal males, (○--------○); normal females, (■—■).

Fig. 9 indicates the response of experimental and control groups of mice. Thymectomy of normal male mice enhanced the response to PVP, confirming previously published data (22). Hence, castration and thymectomy which also generates enhancement should have produced an additive enhanced effect. It did not. In fact, thymectomy abolished the enhancement seen after gonadectomy (Fig. 9 B).

In order to test the environment in castrated vs. normal male mice, animals were lethally irradiated and reconstituted with 10 million normal spleen cells and 5 million bone marrow cells. Separate groups of animals were immunized with 0.1 μg of PVP intravenously or with either 0.1 cc of 2.5% rat or goose erythrocytes. Fig. 10 indicates the responses to PVP, the thymus-independent

antigen. It may be seen that the castrated, lethally irradiated mice possessed an improved environment for response to this antigen, in that levels of antibody in the castrated group greatly exceeded the serum levels of normal, lethally irradiated reconstituted animals. In contrast, no difference was observed between castrated and normal, lethally irradiated recipients in responsiveness to the thymus-dependent antigens, goose, or rat erythrocytes.

Effect of Gonadectomy of the Male on Stem Cell Differentiation.—In order to evaluate whether the environment was capable of generating differences in stem

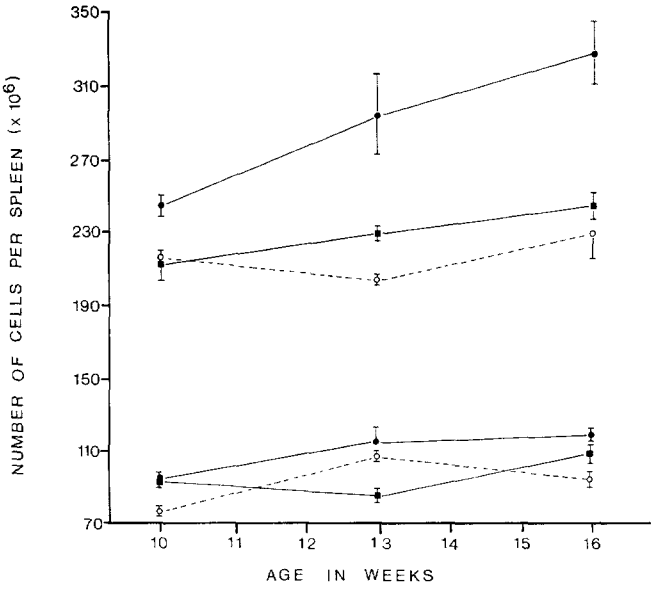

FIG. 8. Nucleated spleen cell counts before (upper set of curves) and 48 hr after (lower set of curves) administration of cortisone acetate in a dose of 125 mg/kg body weight. Each point represents mean and SE of 12 B6D2F$_1$ mice per group. Castrated males, (●—●); normal males, (○--------○); normal females, (■—■).

cell differentiation which could account for the enhanced responsiveness on the basis of a greater stream of cells differentiating into the lymphoid system from bone marrow multipotent precursor cells, initial evidence for a potential alteration in stem cell differentiation was sought. In these experiments, the capacity for hematopoiesis in reconstituted animals was evaluated, only in the context of an index of potential alterations.

The number of spleen colonies in animals receiving 10^5 bone marrow cells after lethal irradiation was determined. Table II indicates the data of four experiments from which it may be seen in three of these experiments, that the number of colony-forming units in the spleen of reconstituted gonadectomized mice was significantly higher than normal male mice. In one of the four ex-

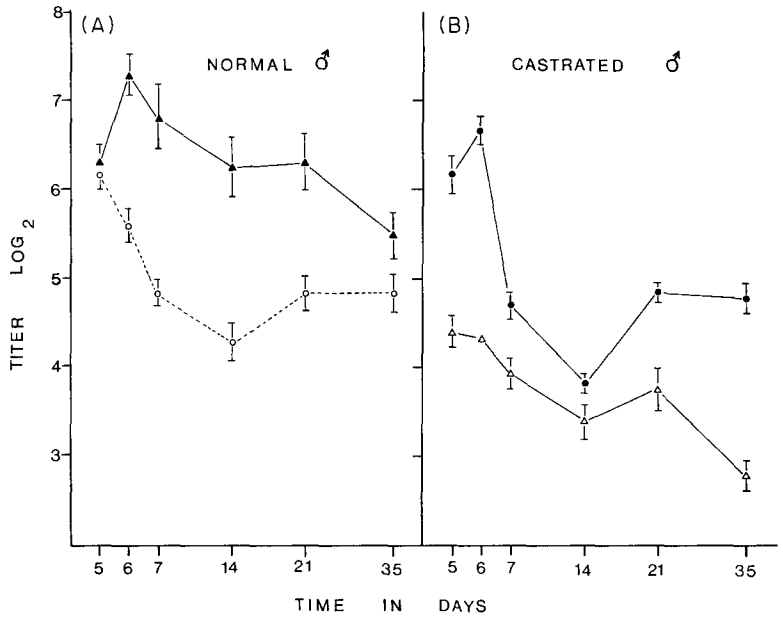

FIG. 9. Primary immune response after administration of 0.1 μg of PVP in various groups of B6D2F$_1$ mice. Each point represents mean and SE of 8–10 mice per group. (A) Thymectomized males, (▲—▲); normal males, (○--------○). (B) Castrated males, (●—●); castrated, thymectomized males, (△—△).

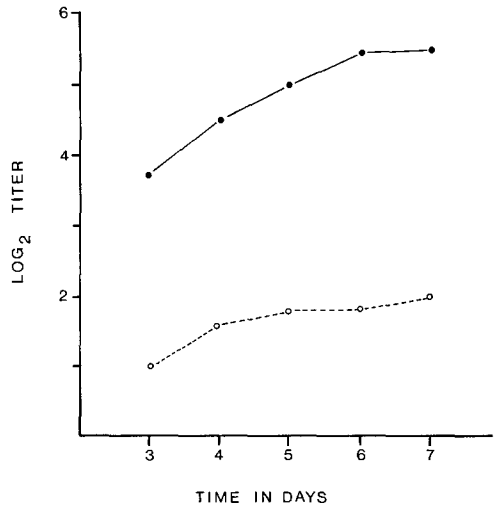

FIG. 10. Response to 0.1 μg of PVP of castrated and normal lethally irradiated CAF$_1$ mice reconstituted with 10^7 spleen cells and 5×10^6 bone marrow cells from normal syngeneic male donors. Each point represents the mean of 10–12 mice. Castrated males, (●—●); normal males, (○--------○).

periments it was noted that the numbers of colonies in the female group were equivalent to those of the normal male mice, and fewer in number than the orchiectomized male animal. The validity and reproducibility of this observation was not tested further.

TABLE II

Splenic Colony-Forming Units in Lethally Irradiated Castrated and Normal Males and Females Reconstituted with 10^5 Bone Marrow Cells

Exp. No.	No. of colonies/spleen		
	Females	Males	Castrates
I	13.7 ± 1.8 (14)	13.3 ± 0.8 (16)	23.4 ± 2.7 (14)
II	—	15.4 ± 1.2 (14)	23.1 ± 1.9 (13)
III	—	18.4 ± 1.9 (5)	34.5 ± 2.5 (4)
IV	—	20.9 ± 2.2 (10)	21.5 ± 2.0 (12)

Numbers in parentheses indicate number of animals employed in each experimental group.

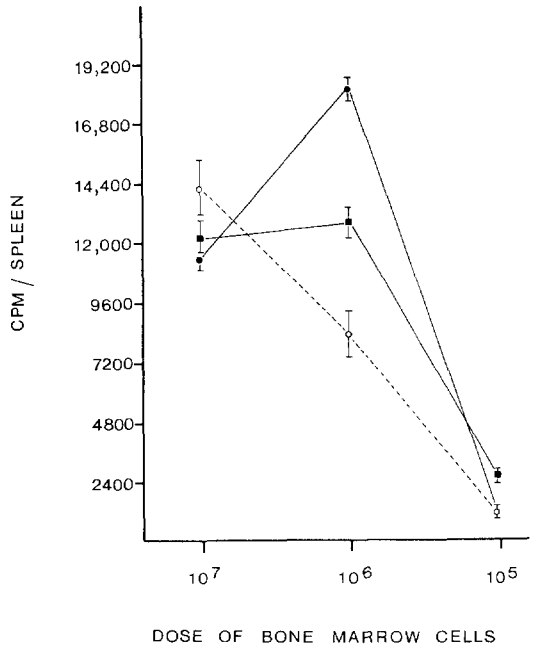

FIG. 11. Uptake of ^{59}Fe expressed as counts per minute (CPM) per spleen taken from normal and castrated males and females. Mice were lethally irradiated and reconstituted with either 10^5, 10^6, or 10^7 syngeneic bone marrow cells. A dose of 0.2 μc of ^{59}Fe was administered intravenously 16 hr before sacrifice, 7 days after lethal irradiation and reconstitution. Each point represents the mean and SE of 12 $B6D2F_1$ mice. In this experiment mice were 12 wk of age, hence, 6 wk after orchiectomy. Castrated males, (●—●); normal males, (○--------○); normal females, (■—■).

In three separate experiments designed to provide more quantitative objective data, a tracer dose of iron-59 was administered and the isotope uptake of growing hematopoietic cells evaluated 1 wk after reconstitution with 10^5, 10^6, or 10^7 syngeneic bone marrow cells. Fig. 11 indicates the data in one experiment of three identical experiments carried out to evaluate this parameter. In two experiments the uptake of radioactive tracer at the dose of 10^6 bone marrow cells was greatest in the castrated male animal. In one case the female exhibited the highest uptake. However, in all three instances the splenic uptake in these two groups of mice exceeded levels of radioactivity in normal male mice. It was believed but not proven that the variation at the 10^7 dose was due to spatial interference induced by reconstitution with excessive numbers of cells.

DISCUSSION

The results of the present work confirmed the experiments of numerous investigators in denoting the enhanced capacity for humoral antibody formation in female vs. male mice. Both IgM (19S) production, particularly in primary immune responses, as well as IgG (7S) antibody production denoted in secondary and tertiary immune responses were enhanced. Thus, under diverse experimental and natural conditions the female may generate enhanced immunoglobulin production in probably all classes of antibody (1–6).

The unusual observation in the present work was the markedly enhanced responses to the thymus-independent antigen, PVP, in female and in castrated male mice. Whereas the responses to thymus-dependent antigens in the castrated mice simply converted the male to the female animal in terms of responsiveness, castrated mice not infrequently exhibited the highest responses to thymus-independent antigens. The marked hyperplasia of the thymus gland after castration at 6 wk of age pointed to this organ as the etiological factor in generating the enhanced responses. This view was confirmed by experiments combining thymectomy with gonadectomy. The enhanced response to PVP in the castrated mice was abolished by thymectomy. Since thymic function diminishes with increasing age (25, 26), the reduction or disappearance of the enhancement with time after castration at 6 wk of age, and the failure to demonstrate enhancement 10 wk after castration of 9- or 10-month old animals, also provided indirect evidence for the role of the thymus is mediating the castration effect. Similar arguments may be put forth denoting the role of the thymus in producing the differential enhanced responses in normal female vs. male mice.

It would appear that the thymus gland is capable of enhancing humoral antibody formation by at least two differing mechanisms. The first is via production of enhancing factor or hormone (27–29), which may or may not be equivalent to thymosin (30). The second mechanism has been more recently described by ourselves (31) and by Gershon and colleagues and others (32–34), namely, that the thymus possesses, at the very least on a functional basis, a population of inhibitory or suppressor cells. The enhanced response to thymus-independent antigen has been interpreted to represent enhanced B lymphocyte

function generated by loss of thymic inhibitory cell function (21). The abolition of the enhancement effect of castration by thymectomy suggests therefore that the enhancement effect in the castrated mice is mediated by thymic hormone. Further evidence that the enhancement is hormonal in origin was derived by comparing responses of lethally irradiated castrated and normal male mice. The response in the former greatly exceeded the latter. Although the thymus is markedly radiosensitive (35), it may be that the thymic hormone production is a radioresistant capability of thymus for which there is some evidence (29), or alternatively, that higher serum levels of the hormone may be reached in the castrated mice before lethal irradiation. Thus, in an absolute sense, the immune response to PVP may be looked upon as being negatively thymus dependent.

A possible role of the pituitary gland was studied indirectly as follows. The response to PVP was evaluated at intervals after oophorectomy of female mice. No enhancement of the response to PVP was seen, indicating that the pituitary-endocrine axis is not involved in the enhanced response. Thus, enhanced pituitary hormonal activity after gonadectomy does not mediate the effect.

The substantial increase in cortisone-sensitive cells in the thymus after castration, while preserving equivalent numbers of cortisone-resistant cells, suggests that there may be a greater production of thymic lymphocytes from precursor cells after castration. The observed enhancement of the secondary as well as the primary immune responsiveness to the highly thymus-dependent antigens, horse, or goose erythrocytes suggests that the cellular differentiation of T1 to T2 lymphocytes (36) may also be promoted. That the effect on thymus-dependent antigens is mediated by cellular rather than environmental factors is indicated by the absence of enhanced response to goose or rat erythrocytes in lethally irradiated and reconstituted castrated mice when compared with ethally irradiated and reconstituted normal male mice. Thus, it is the cell inoculum rather than the host environment (in contrast to thymus-independent antigen) which is the overriding factor in enhanced response to thymus-dependent antigens.

The observed increases in numbers of colonies and ^{59}Fe uptake of hematopoietic cells in the spleens of bone marrow-reconstituted female or gonadectomized male animals as compared to normal males provided indirect evidence that stem cell differentiation, at least in the hematopoietic system, is enhanced in an environment deficient in androgenic hormone. It may be more than coincidental that the reconstitutive capacity of bone marrow is directly proportional to the thymus weight of the three groups of mice. Neonatal thymectomy has been shown to diminish numbers of spleen colonies (37), while addition of syngeneic thymus cells to a bone marrow inoculum augments numbers of hematopoietic colonies (38). However, the potential role of trophic hormones derived from the pituitary gland was not evaluated by experiments employing oophorectomized females, nor was stem cell differentiation in the lymphoid system examined directly at this time. Nevertheless, all of the findings taken

together imply a profound albeit complex influence of the sex hormones on cell proliferation and differentiation in both the lymphoid and hematopoietic systems, mediated both by hormonal or environmental, and cellular mechanisms.

The enhanced capability of hematopoietic stem cell differentiation from bone marrow precursors in castrated animals raises serious questions regarding the clinical use of androgens to promote stem cell differentiation in aplastic anemia and myelofibrosis. Its use would seem in part to be related to the observation of the stimulation of erythropoiesis in rodents (39, 40) brought about (perhaps) by increased levels of erythropoietin (41, 42). There is no doubt that androgens can induce a remission in patients with aplastic anemia (43, 44) or myelofibrosis (45). However, evidence for a direct effect of androgens in stimulating erythropoiesis is controversial, some workers being in favor (46, 47) and some opposed (48, 49). The observations in the present work imply that the mechanisms of stimulation of erythropoiesis are complex, and that the role of androgens in the clinical treatment of aplastic anemia and myelofibrosis need to be reassessed.

SUMMARY

The primary and secondary immune responses to thymus-dependent and -independent antigens were evaluated in normal male and female mice and in castrated male mice. Both IgM antibody production in the primary response and IgG antibody production in the secondary response were enhanced in females vs. males of equivalent age. Castration of the male converted this animal to a female in terms of responsiveness to the thymus-dependent group of antigens, while inducing equivalent or even greater enhanced responsiveness over the female to the thymus-independent antigen, polyvinylpyrrolidone.

Further characteristics of the changes in lymphoid organs were determined in the castrated animal vs. normal males and females. It was shown that the spleen and thymus became markedly hyperplastic, the organ weights exceeding the female, which in turn were greater than in the male. The enhanced weight of the thymus was shown to be due to increased numbers of cortisone-sensitive cells, the absolute number of cortisone-resistant cells remaining equivalent to normal males and females. Thus, the increased thymic weight of the female also resided in the cortisone-sensitive population. Peripheral lymphocyte counts in castrated animals exceeded both normal males and females. Further experiments in gonadectomized males provided evidence that increased thymic cell activity per se played a role in enhanced response to thymus-dependent antigens, but that a thymic-derived hormone mediated the enhanced effect to the thymus-independent antigen in the castrated animal. The capacity for loss of androgenic hormone-producing tissue to generate enhanced differentiation of stem cells was denoted by experiments in which numbers of spleen colonies and uptake of ^{59}Fe, employed as an index of hematopoiesis 1 wk after reconstitution of lethally irradiated castrated and normal recipients, were enhanced in gonadectomized male animals. Thus, in summary, changes in sex hormone levels exerted a marked influence on immune responsiveness and stem cell

differentiation, by increasing numbers of functioning cells, by promoting cellular differentiation, as well as by promoting cellular function via hormonal effects.

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