REGULATION OF IMMUNOGLOBULIN SYNTHESIS BY DEXTRAN*

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Stimulation of lymphoid cells is now a well established effect of such mitogens as endotoxic lipopolysaccharides [LPS, cf. (1)],[1] concanavalin A [Con A, (2, 3)], phytohemagglutin [PHA, (4)], and pokeweed mitogen [PWM, (4–6)]. Through their actions on lymphoid cells these substances have been most useful for elucidating certain aspects of the mechanism of immunological reactivity. For instance, certain of the endotoxins have been characterized as improving humoral antibody synthesis towards other antigens both in vivo (7–9) and in vitro (10, 11). More recently LPS have been shown to heighten the functions of bone marrow (B) but not thymic (T) cells (12). Indeed, the T cell requirement in the IgM anti-SRBC hemolytic response of the mouse has been revealed to be replaceable by LPS (12, 13).

Conversely, the stimulatory activities of Con A and of PHA are restricted primarily to T cells (2–4, 14, 15) although Con A can be made to activate B cells for immunoglobulin synthesis by making Con A insoluble (4).

PWM on the other hand, may exert its activity upon both B and T cells (5). Its selective stimulation of IgM synthesis by mouse B lymphocytes has been dissociated from any antigenic property of the mitogen and has been characterized as an "aspecific" phenomenon (6).

Purified protein derivative of tuberculin (PPD) has been thought to cause blastogenesis of essentially thymus-derived cells when these originate from PPD-sensitive individuals (16–18). In the nonimmune condition, however, PPD has been described as an effective B cell mitogen as well as a stimulator of B cells for antibody synthesis to other nonrelated antigens (19, 20).

We have recently shown that dextran, a substance normally not considered to be a mitogen, alters immunological responses to heterologous antigenic and haptenic stimuli (21). In guinea pigs, low molecular weight dextran postponed delayed dermal hypersensitivity until a later time when skin responses were greatly exacerbated. In addition, pretreatment with this polysaccharide de-

pressed the synthesis of circulating antibody that mediates passive cutaneous anaphylaxis (PCA). On the other hand, if infused into guinea pigs with established immunological responses dextran did not alter either delayed hypersensitivity or PCA antibody synthesis (21).

In view of dextran's clinical importance as a blood plasma expander and as an ingredient of certain medications, we thought it appropriate to ascertain the breadth of applicability of its effects. In particular, we wished to know whether it disrupted the normal course of immunological responses such as IgM and IgG synthesis. Further, we wished to determine if any immunological alterations were detectable at a cellular level where the possibility exists for elucidating certain events associated with the mechanisms of immunological responsiveness and unresponsiveness.

Materials and Methods

Dextran Treatment of Guinea Pigs.—Guinea pigs, of both Hartley (The Rockefeller University, New York) and Pirbright (Animal Virus Research Institute, Pirbright Woking, Surrey, England) strains, were intrajugularly injected with 300 mg of dextran (Pharmacia Fine Chemicals, Inc., Piscataway, N. J., clinical grade H lot no. H1763, average molecular weight 186,000) 6 or 24 h before introducing antigen. The latter was a 0.25% sheep red blood cell (SRBC) suspension in 1 cc volume given intraperitoneally. Control animals received SRBC only.

Plaquing.—At 4.5 days after injecting SRBC, animals were sacrificed, their spleens removed, and cells separated by passage through nylon mesh. It is noteworthy to mention that most all of the determinations of plaque-forming cells (PFC) on spleens of guinea pigs were done at 4.5 days, a time that is near maximal for IgM and IgG PFC in this animal (22). Cells were counted, washed, reconstituted to suspensions of 10^5 and 10^6 per cubic centimeter using Earle's balanced salt solution (Grand Island Biological Co., Grand Island, N. Y.). The Jerne method (23) of plaquing was used to determine IgM and the Wortis modification (24) was used for identifying IgG. Rabbit antibody to guinea pig γ-globulin (1:50) was used to detect IgG synthesis and pooled guinea pig sera (1:15) was the source of complement. Total time of incubation was 3 h at 37°C in a 5% CO_2 atmosphere.

Treatment of Controls.—To test for cross-reactivity of dextran to SRBC, guinea pigs were inoculated intravenously with 300 mg of dextran and their spleen cells plaqued against SRBC at 4.5 days.

To determine whether a booster effect was being achieved by administering dextran before SRBC, some animals were given two injections of SRBC 6 h apart and their spleen cells were plaqued at 4.5 days.

To determine if endotoxin would mimic the dextran effect, 100 μg of *Escherichia coli* lipopolysaccharide (055:B5 Difco Laboratories, Detroit, Mich.) in 2 ml volume was injected intravenously at either 6 or 24 h before SRBC, and spleen cells of these animals were plaqued at the usual 4.5 days.

Spleen cells from untreated animals were used to measure background counts.

Dextran Treatment of Mice.—Mice used were CBA/Wh and CBA/J. Injections of dextran (amounts stipulated in tables were contained in 0.3 cc volume) were via the retro-orbital sinus at indicated intervals before antigen (SRBC). Plaquing routinely occurred at 4.5 days after intraperitoneally administered antigen. For developing indirect PFC rabbit antimouse IgG (Cappel Laboratories, Downingtown, Pa.) was used.

Dextran's effect on B and T cells was determined by first removing the B and T cells from

untreated mice. B cells (10^7) alone or B (10^7) and T (20×10^6) together were then injected intravenously into X-rayed (900 R) syngeneic recipients. Representatives from both groups were treated with dextran (12 mg) 24 h before SRBC. The remainder were given only SRBC. Plaquing as before was done at 4.5 days.

The effect of dextran on T cells alone was done in two ways. In the first method, T cells (80×10^6) from donor animals were transferred to X-rayed recipients. 12 mg dextran was injected intravenously into each animal and 24 h later spleens removed, cells separated, mixed with untreated B cells (10^7 B and 20×10^6 T), and injected into X-rayed recipients. SRBC were given intraperitoneally 24 h later and plaquing was done at 4.5 days.

The second method used was an in vitro treatment of T cells with dextran. A mixture containing T cells, 5% mouse serum, 1% antibiotic (penicillin-streptomycin, Grand Island Biological Co.), and dextran (0.1–1 mg/1 cc) was incubated for 2 h at 37°C. Cells were then spun, supernatant removed, and cells brought to a concentration of 20×10^6 in 0.1 cc, mixed with normal B cells (10^7 per 0.1 cc) and injected into X-rayed recipients. Total amount of cells given one animal was 40×10^6 T and 20×10^6 B. SRBC were given 24 h later and spleen cells plaqued at 4.5 days.

Culturing Cells In Vitro.—For guinea pigs the preparation, culture, and harvest of lymphocytic cultures were done as has been described (25).

For mice, essentially the same procedures were maintained except that the culture medium was RPMI-1640 (Grand Island Biological Co.) to which was added antibiotics, glutamine, and fetal calf serum.

The dextran added was the same as that used for the other experiments and the concentrations were those indicated in Table IV.

RESULTS

The guinea pig is a particularly good animal species in which to observe the alterations of immunological reactions that are inducible by dextran. Typically, one dose of dextran intravenously administered before an intraperitoneal injection of SRBC caused a profound increase in the synthesis of IgM to SRBC. Whereas guinea pigs given SRBC alone possessed 10 direct plaque-forming cells (DPFC) per million spleen cells, animals pretreated with dextran 6 h before SRBC had 20 times this number (Table I). Allowing 24 h to elapse between the injections of dextran and SRBC caused a sevenfold increase in DPFC per million. The total number of DPFC in the spleens of these animals also reflected the dextran effect since a 46-fold improvement occurred at the 6 h pretreatment and a sevenfold improvement was apparent at the 24 h interval (Table I). Thus, IgM synthesis in guinea pigs would appear to be optimally altered by the shorter rather than the longer interval allowed between administering dextran and antigen.

Synthesis of IgG in guinea pigs was also markedly increased by the polysaccharide, but in a different time sequence. For this particular immunoglobulin, the longer 24 h interval between dextran and antigen administration favored production of IgG. Thus, whereas a threefold increase in indirect plaque-forming cells (IPFC) per million spleen cells assayed was noted at the 6 h interval (Table II), a 39-fold increase was apparent at the 24 h period. In parallel, the total number of IPFC per spleen was greater at 24 h (28 times normal) than at 6 h (6 times normal, Table II). By giving dextran 48 h before

TABLE I

Dextran-Induced Alterations in IgM Synthesis by Guinea Pigs to SRBC

Pretreatment	Antigen	No. of guinea pigs	DPFC*	
			Per 10⁶	Per spleen
None	SRBC	5	10	5,204
Dex‡ 2 h before	SRBC	2	5	1,714
Dex 6 h before	SRBC	2	204	238,666
Dex 24 h before	SRBC	2	70	34,822
Dex 48 h before	SRBC	2	54	29,119

* Detected 4.5 days after injection of SRBC.
‡ 300 mg dextran per kilogram body weight.

TABLE II

Increased IgG Synthesis to SRBC in Guinea Pigs Attributable to Dextran

Pretreatment	Antigen	No. of guinea pigs	IPFC*	
			Per 10⁶	Per spleen
None	SRBC	5	3	1,995
Dex‡ 2 h before	SRBC	2	2	1,336
Dex 6 h before	SRBC	2	9	11,439
Dex 24 h before	SRBC	2	117	55,880
Dex 48 h before	SRBC	2	0	0

* Detected 4.5 days after injection of SRBC.
‡ 300 mg dextran per kilogram body weight.

antigen, IgG antibody synthesis ceased whereas IgM antibody production continued. Thus, simply by pretreating with dextran, an unusually greater synthesis of IgG to SRBC can be made to occur in guinea pigs at a time that is ordinarily not optimal for this species.

Certain controls essential for establishing the manner in which dextran exerts its influence upon immunological responses are presented in Table III. To ascertain the background count of cells producing antibody to SRBC, spleen cells from unimmunized guinea pigs were examined. Approximately the same number of DPFC was observed in animals immunized intraperitoneally with one dose of SRBC at 4.5 days as was seen in control animals given no SRBC. A small number of IPFC were seen in immunized guinea pigs that were not detectable in unimmunized animals.

Two sorts of tests were made to determine whether dextran possesses antigenic cross-reactivity with SRBC. In the first, guinea pigs were injected with dextran alone and their spleen cells were examined 4.5 days later for PFC toward SRBC. As shown in Table III, the number of DPFC and IPFC paralleled those of unimmunized animals. In the second test, five different concentrations of dextran (1.0 to 0.0001 mg/ml) were incubated with equal volumes

TABLE III

Pertinent Controls for Establishing the Immunological Changes Induced by Dextran

Pretreatment	Antigen	No. of guinea pigs	DPFC*		IPFC*	
			Per 10^6	Per spleen	Per 10^6	Per spleen
None	None	3	10	3,414	0	0
None	SRBC	5	10	5,204	3	1,995
Dex‡	None	2	12	6,488	0	0
SRBC 6 h before	SRBC	1	11	4,262	4	1,550
Endotoxin§ 6 h before	SRBC	1	6	3,936	0	0
Endotoxin, 24 h before	SRBC	1	14	8,352	0	0

* To SRBC detected 4.5 days after injection of SRBC.

‡ 300 mg dextran per kilogram body weight.

§ From *Escherichia coli*; 100 µg per animal (lethal dose for 50% of the animals approximates 500 µg per kilogram).

of antibody to SRBC (1:300 dilution of antiserum possessing endpoint hemagglutination titer of 1:900) before the addition of appropriate amount of SRBC (0.25% suspension). After suitable incubation no inhibition of the agglutination was observed (these data are not given in the tables). By these two criteria it was concluded that dextran is not antigenically cross-reactive with SRBC.

To find out if the dextran pretreatment of guinea pigs was acting in a manner akin to the first injection of antigen in a booster-type immunization, a guinea pig was injected twice with SRBC 6 h apart. The number of DPFC and IPFC (Table III) was not appreciably different from those of animals injected once with SRBC, thus suggesting that dextran's influence is not that of a booster-effect in the classical sense.

Whether an endotoxin-type of adjuvanticity could explain dextran's activity on immune responses was examined by pretreating guinea pigs with an endotoxin preparation from *Escherichia coli*. A dose of 100 µg per animal given either 6 or 24 h before SRBC was not able to alter the IgM or IgG responses in a manner comparable to dextran (Table III).

To determine whether dextran's exacerbation of immunoglobulin synthesis is limited to one species of animal or alternatively is a widespread phenomenon, another species was utilized. That the finding is basic to the immunological response mechanism in general may be seen from the data gathered as well in the mouse (Table IV). The number of DPFC per million was not significantly different from the control value when dextran preceded antigen by 2 h. Thereafter the DPFC increased until a maximum number was attained when the interval between dextran and antigen was 24 h.

The IPFC response was the mirror image of the DPFC response. A maximal improvement in the number per million (fourfold over the control value) occurred when 2 h elapsed between dextran and antigen. Thereafter the number

of IgG-producing cells decreased as the interval between the two was lengthened (Table IV).

With slight modifications the total number of PFC per spleen conveyed the same impressions (Table IV). Whereas the total number of IPFC paralleled the number per million rather well, two changes were noted in DPFC: a two-fold increase was seen at 2 h and the maximum number appeared at 6 h rather than at 24.

Of particular importance is the fact that in mice pretreated 48 h before SRBC, a six- to sevenfold decrease in IPFC per million and per spleen was observed (Table IV). Thus, depending upon when the polysaccharide is administered relative to antigen it can cause an increase as well as a decrease in the number of cells synthesizing IgM and IgG antibody.

TABLE IV

Heightened IgM and IgG Synthesis as well as Depressed IgG Production to SRBC in Mice Evoked by Polysaccharide Pretreatment

Treatment of mice		No. of mice	Direct PFC‡		Indirect PFC‡	
Dextran*	Antigen		Per 10⁶	Per spleen	Per 10⁶	Per spleen
None	SRBC	8	146	12,701	282	23,282
2 h before	SRBC	4	124	24,304	1,000	196,000
6 h before	SRBC	3	273	52,219	713	136,874
24 h before	SRBC	6	310	33,016	311	34,983
48 h before	SRBC	4	288	36,186	40	3,485

* 12 mg of dextran intravenously.

‡ Detected 4.5 days after injection of SRBC.

To establish the optimally effective dose of dextran necessary to induce heightened immunological responses in guinea pigs and mice, groups of animals were injected with varying doses of dextran 6 h before SRBC. Spleens were assayed for DPFC and IPFC 4.5 days later. A dose of 3 mg/10 g body weight was maximally operative for increasing DPFC in guinea pigs while 1 mg/10 g body weight was effective for both DPFC and IPFC in the mouse. With larger doses only a slight decline in stimulatory effectiveness occurred (Figs. 1 and 2).

One of the more direct explanations for the mechanism by which dextran exerts its effect upon the immunological system is to assume it provides a stimulus for cells to divide. This possibility was examined both in vivo and in vitro. Two in vivo tests were made in mice and one in guinea pigs. In the first, mice were given dextran intravenously (5 mg/10 g) and their spleen cells were counted at various intervals thereafter. A twofold improvement in the total cell counts was observed at 4.5 days. In the second, mice were irradiated with 900 R and then given fixed numbers of B cells, T cells, or B and T cells. Each group was then divided in half and one-half the animals were given
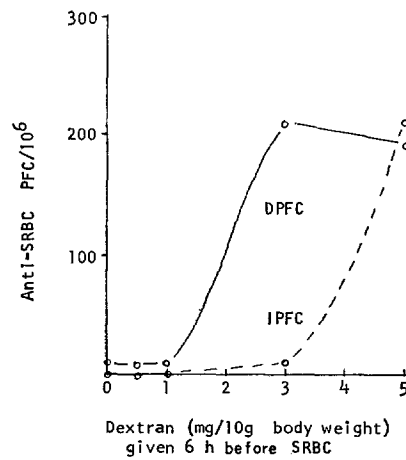
FIG. 1. Optimal dose of dextran that alters immune responses of guinea pigs.
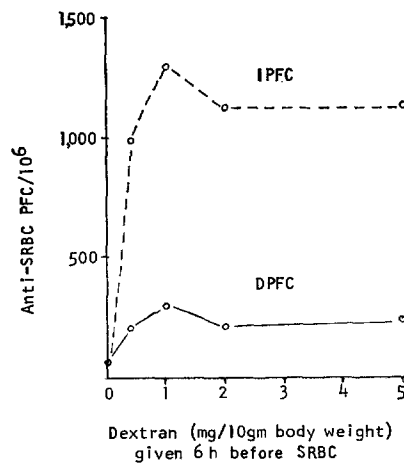

FIG. 2. Optimal dose of dextran that alters immune responses of CBA mice.

the standard dose of dextran. Counts were made of the cells in spleens at 24 h and at 4.5 days. Again approximately a twofold rise over background controls was apparent in each cell type as well as in the mixture at both time intervals.

The in vivo test in guinea pigs was conducted just as was the first in vivo test in mice except that the dose of dextran given was 3 mg/10 g body weight. Just as with the mice a twofold increase in spleen cells was noted at the 4th to 5th day.

For in vitro tests, the lymph node cells of a normal guinea pig and the bone marrow, thymic as well as spleen cells of a mouse were grown in the presence of 10 μg, 100 μg, and 500 μg of dextran. Each tube was pulsed with radioactive

thymidine 24 h before harvesting and the amount of label retained by the cells was ascertained by liquid scintillation counting. Over the entire 96 h dextran provided no appreciable stimulus to any of the cells from either species of animal to undergo blastogenesis that differed significantly from control cultures (Table V).

To ascertain at what level in the immunological system dextran exerts its effect, B and T cells were treated separately both in vivo and in vitro with dextran before immunization. In the first experiment CBA mice that had been irradiated with 900 R were given 20×10^6 B cells. A number of the cell recipients were then treated intravenously with dextran (5 mg/10 g) and 24 h

TABLE V

Inability of Dextran to Induce Blastogenesis of Nonimmunized Lymphoid Cells Grown In Vitro

Animal species	Cells (no. $\times 10^{-6}$)	Dextran	CPM*		
			48 h	72 h	96 h
		µg			
Guinea pig	Lymph node	None	1,172	804	752
	(10)	10	821	775	660
		100	833	554	589
		500	911	702	939
Mouse	Bone marrow	None	5,822	4,950	ND‡
	(2)	10	5,990	4,783	
		100	6,242	5,175	
	Thymus	None	1,122	1,040	ND
	(2)	10	1,705	903	
		100	1,431	1,138	
	Spleen	None	4,715	12,658	ND
	(2)	10	4,077	14,374	
		100	3,892	11,214	

* Mean counts per minute of duplicate or triplicate cultures.
‡ ND, not determined.

later both treated and untreated animals were immunized with SRBC. As expected, mice receiving B cells and SRBC only showed the background number of DPFC (12 per million) and no IPFC (Table VI).

In contrast, B cell recipients that had been pretreated with dextran 24 h before SRBC showed the full number of DPFC (31 per million) that is normally seen when B and T cells are given together. In concert with the untreated mice, no IPFC were observed in dextran-treated B cell recipients. Ordinarily, no IPFC are seen in B and T cell recipients at 4.5 days of immunization with SRBC.

Two experiments were conducted to determine whether dextran affects the activity of T cells. In one, T cells were simply incubated for 2 h in vitro in Earle's balanced salt solution containing dextran (1 mg/ml). The T cells were thereafter washed in excess Earle's BSS and, in combination with normal un-

TABLE VI

IgM Synthesis at 4.5 days: Substitution of T Cells' Effect upon B Cells by Dextran.
IgG Synthesis at 4.5 days: Requirement for T Cells' Effect upon Dextran-Primed B Cells

Cells transferred	Treatment of cell recipients	No. of recipients	No. per million (±SD)	
			DPFC	IPFC
B only	No dextran; SRBC	6	12 (5)	0
B only	Dextran 6 or 24 h pre-SRBC	8	31 (10)	0
B + T	No dextran; SRBC	2	39 (20)	0
T (dextran-treated) + normal B	No dextran; SRBC	6	43 (17)	0
B + T	Dextran 6 or 24 h pre-SRBC	6	52 (30)	58 (58)
B (dextran-treated) + normal T	No dextran; SRBC	5	38 (33)	33 (33)

treated B cells, injected into X-irradiated recipients that were then immu-
nized with SRBC. These animals demonstrated the number of DPFC (36 per
million) that normally is seen when these two types of cells cooperate. They
also did not demonstrate IPFC which again is a normal event at this immuniza-
tion period. These data are not given in the table.

In the second experiment T cells were treated in vivo with dextran. In this
case 80 × 10⁶ T cells from normal CBA mice were given each X-rayed re-
cipient and half of these animals were treated intravenously with 12 mg dex-
tran. 24 h later the animals of both sets were sacrificed, their spleens removed,
and cells expressed. The T cells from spleens, after washing and counting,
were mixed with normal B cells (10 × 10⁶ B + 20 × 10⁶ T) for injection into
new X-rayed animals that were subsequently immunized with SRBC. The
number of DPFC for both groups of mice was the same as seen previously (43
per million) and there were no IPFC (Table VI). These two experiments suggest
but do not prove that dextran may have no direct effect upon T cells.

To further determine the level of dextran's activity, B and T cells from nor-
mal donors were given to X-rayed recipients (20 × 10⁶ and 40 × 10⁶, re-
spectively) and the latter were treated with dextran 24 h before injecting the
antigen, SRBC. Not only was the normal number of DPFC attained (52
per million) but, in addition, IPFC became evident for the first time (58 per
million). Thus, the T cell is a necessary requirement for causing dextran-
primed B cells to synthesize IgG earlier in the immunization schedule.

Nevertheless, it remained unclear whether T cells must also be affected by
dextran to allow IgG synthesis to proceed at this early stage. A further ex-
periment involving treatment of B cells alone with dextran and mixing these
with normal T cells was required to clarify this point. B cells (50 × 10⁶)
were given each of several X-irradiated mice and the next day they were given

dextran (5 mg/10 g) intravenously. 2 h later they were sacrificed, their spleens removed, and cells expressed. These B cells, after washing and counting, were mixed with normal T cells (20×10^6 B $+ 40 \times 10^6$ T) for injection into new X-rayed animals that were subsequently immunized with SRBC. The number of DPFC and IPFC approximated those seen when B and T cells were given animals that had received dextran 6 or 24 h before SRBC. This experiment also suggests yet does not prove that dextran acts primarily upon B cells and not upon T cells. A rigorous proof necessitates removal of the small number of T cells known to be present among bone marrow cells.

<div align="center">DISCUSSION</div>

Administering dextran to guinea pigs and mice caused marked alterations in their abilities to synthesize IgM and IgG to heterologous antigen, SRBC (Tables I, II, and IV). The minimal dose that produced a maximal stimulating effect on antibody synthesis was 3 mg/10 g of body weight for guinea pigs (Fig. 1) and 1 mg/10 g of body weight for mice (Fig. 2). However, once achieved the maximum effect was observed over a rather wide dose range in each species.

To determine whether improvement in antibody synthesis was attributable to antigens held in common by dextran and SRBC, three tests were made. Dextran, when given alone as antigen to guinea pigs, was unable to stimulate an increased number of SRBC-specific PFC of either the direct or indirect variety (Table III). In the second test, incubating dextran in several concentrations with SRBC-specific antibody did not inhibit agglutination of SRBC. Furthermore, administering SRBC instead of dextran before the regular injection of SRBC did not mimic dextran's mobilization of IgM and IgG production (Table III). Finally, we have reported elsewhere that dextran alters immunological responses of guinea pigs to stimuli other than SRBC, i.e., bovine gamma globulin and picryl chloride (21). Thus, the described dextran activation is not attributable to cross-reactive antigenic prestimulation of antibody-producing cells.

Whether the effect could be assigned to an endotoxic quality of the dextran preparation appeared, at the outset, unlikely. This was inferred since antibody stimulation by dextran was achieved by giving dextran before antigen whereas endotoxin has been reported to be effective when given after antigen (10). Nevertheless, to investigate the possibility, endotoxin from *Escherichia coli* was given to guinea pigs before SRBC. When the numbers of DPFC and IPFC from these animals were seen to be similar to those of nontreated control animals, this possibility was dismissed (Table III).

One mechanism by which dextran stimulates the immunological apparatus could conceivably be by causing lymphoid cells to divide. This idea was examined exhaustively in the two animal species both in vivo and in vitro. Dextran given in vivo was able to cause the number of viable lymphoid cells present in the spleens of both sorts of animals to increase twofold. This result

was observable 4 to 5 days after giving dextran. In addition, both B and T cells separately transferred to X-irradiated mice, showed a twofold increase in number in those mice given dextran.

Actually, a twofold division of spleen cells appears unlikely to account for the large increase in SRBC-specific PFC. Only if the replication were a selective one among certain clones of cells might many more divisions have occurred while maintaining an overall appearance of a twofold increase. Yet in vitro the lymph node cells of guinea pigs and the bone marrow, thymic, and spleen cells of mice revealed no dextran-induced blastogenesis as measured by thymidine uptake (Table V). Thus, if the in vivo replication is to be considered significant, dextran administration must be assumed to have elicited the appearance of an intermediary product in vivo capable of inducing blastogenesis. However, since B cells exposed in vitro to dextran were able, upon transfer to X-rayed mice, to show a heightened immune response to SRBC (Table VI), it is unlikely that dextran acts primarily in a mitogenic manner. In this respect it is unlike PPD, PWM, and LPS, all of which induce B cell blastogenesis (3, 5, 19).

For any explanation of the mechanism by which dextran alters immunological responses, knowledge of its effects at the cellular level becomes necessary. By treating B and T cells separately with dextran both in vivo and in vitro, and then transferring them to X-rayed animals that were subsequently immunized, it was found that B but not T cells were primarily affected by dextran. Actually, exposing B cells to dextran either in vitro or in vivo caused DPFC to appear in numbers ordinarily seen only when normal B and T cells act in concert. Although dextran's action would thus appear to be primarily upon B cells, it could conceivably be directed to the small number of T cells known to be present in bone marrow preparations. Experiments to remove these T cells with anti-θ antibodies and complement are in progress. If dextran's effect is solely upon the B cell then it is able to substitute for one function performed by T cells. In this regard it would parallel the action of LPS, PWM, and PPD, all of which stimulate B cells to IgM synthesis in the absence of T cells (6, 12, 13, 20). On the other hand, if dextran acts upon the small number of T cells residing in bone marrow it would be amplifying their effect upon B cells.

As has been reported, B cells alone do not cause the appearance of IPFC to SRBC in vivo (26). Our data (Table VI) support this observation and we further show that B cells primed with dextran still remain unable to cause the appearance in vivo of IPFC. In our experience not even B and T cells together will cause IPFC to SRBC to appear at 4.5 days of immunization. However, mice given B and T cells and subsequently given dextran in vivo do make IPFC at 4.5 days. Indeed, dextran-primed B cells in conjunction with normal T cells will also make the same number of IPFC at this early time. Thus, dextran cannot substitute for T cells in the IgG response. However, by its

interaction with B cells, dextran in conjunction with T cells causes greater numbers of B cells to synthesize IgG earlier than normal (Table VI).

Since the synthesis of antibodies to many polysaccharides has been shown to be T cell independent (27–30), the key to the mechanism by which dextran affects immunological responses toward T cell-dependent antigens may lie in this direction. Thus, an interaction of B cells with a simple polysaccharide, such as dextran, may cause changes (permeability?) such that the cells become receptive to other haptenic or antigenic information. Informed B cells then synthesize the only antibody of which they are capable in the absence of T cells, i.e., IgM. It must be stressed that not all polysaccharides indiscriminately possess this capacity. Pneumococcal polysaccharide Type III injected into mice 3 days before SRBC has been reported not to influence the eventual response to the erythrocytes (31).

In view of the fact that T cells secrete a nonspecific humoral factor that acts on B cells' proliferation or maturation (32–34), it would be interesting to speculate whether that factor is a polysaccharide-containing molecule. An extrapolation of this thought would be to consider whether PPD, PWM, and endotoxins derive their activity upon B cells from contained polysaccharides.

Whether to classify dextran as an adjuvant is problematic. The properties that a substance must possess to allow its consideration as an adjuvant are not perfectly clear. To be sure dextran directly increases the number of B cells synthesizing IgM and, in conjunction with T cells, improves the number of B cells making IgG, but it does so only when given at certain time intervals relative to antigen. For instance, in the guinea pig dextran given 6 h before SRBC caused a marked stimulation of IgM-PFC but when given 24 h before erythrocytes it resulted in heightened IgG-PFC (Tables I, II). Such odd behavior does not make it fit the normal pattern of an adjuvant. In addition, dextran caused a definite dislocation of delayed hypersensitivity responses as well as a depression of PCA-antibody synthesis in guinea pigs (21). These qualities would hardly characterize it as an adjuvant. At the cellular level adjuvants have been shown to alter macrophages so as to markedly improve immune responses (35). It has been suggested that adjuvant increases the effectiveness of the interaction of macrophages and T lymphocytes and that this results in greater stimulation of B lymphocytes to produce antibody (36). This is particularly apparent for IgG and much less a requirement for IgM synthesis (37). Thus, the stimulation of T cells and/or macrophages appears to be an important aspect of the mechanism of action of at least some adjuvants.

Dextran's effect upon macrophages has not yet been investigated in this context. However, since dextran-primed B cells plus normal T cells transferred to irradiated mice resulted in increased numbers of PFC (Table VI), only B

cell-associated dextran could have affected macrophages and T cells. Without doubt, grouping dextran with adjuvants must await further developments.

Since dextran alters cell-mediated and humoral antibody responses, its effects have significance for the mechanisms involving these responses. Two areas beyond those already discussed are particularly pertinent to examine. One involves the shifts in IgM and IgG synthesis while the other concerns factors that activate and inhibit antibody production.

Fig. 3 has been prepared to allow close comparisons of the manner in which dextran influences the shifts in synthesis of IgG and IgM that occur in the two species of animals. At the outset when no dextran but only SRBC was

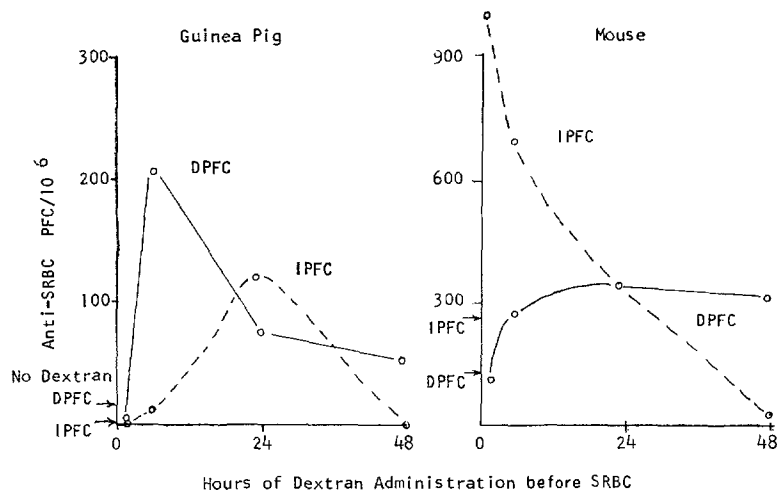Hours of Dextran Administration before SRBC

FIG. 3. Influence of dextran on the shifts in synthesis of IgG and IgM.

given and spleen cells were examined 4.5 days later, the guinea pig had more DPFC than IPFC. The opposite was true in the CBA mouse.

When dextran was given 2 h before SRBC there was a small, almost imperceptible, dip in the number of DPFC in animals of both species. At this time whereas little change was seen in the IPFC of the guinea pig, those of the mouse increased to a maximum. An obvious question to be answered is why the shorter 2 h exposure to dextran should improve IgG synthesis to SRBC so dramatically in the mouse but not the guinea pig. The answer may be that the immunological response to SRBC would appear to be a primary one in the guinea pig but a secondary one in the CBA mouse. In this connection, virgin precursor cells have been reported to have IgM receptors on their surfaces regardless of the class of antibody they eventually secrete. In addition, a temporary change in receptors from IgM to IgG occurred after a single antigenic exposure but prolonged immunization induced a more permanent shift (38).

With a 6 h gap between dextran and SRBC, DPFC attained a maximum

in animals of both species (Fig. 3). With this pretreatment the IPFC of the guinea pig had begun to increase while those of the mouse decreased. Under the influence of dextran it is approximately at this point in time that synthesis of immunoglobulins shifts. The change is from M to G in the guinea pig but from G to M in the mouse. It is not suggested here that dextran initiates the shifts directly. Rather the shifts may be triggered by factors generated in vivo, perhaps by T cells. This is suggested because IgG production in particular does not occur to SRBC in vivo in the absence of T cells (26 and Table VI).

The close synchrony in the shifts of antibody synthesis in animals of the two species suggests that one B cell may be producing both immunoglobulins. This is inferred because a simultaneous increase in cells synthesizing both types of immunoglobulins has not been observed. Rather the production of one increases only when the other decreases.

The maximum number of IPFC was not seen in the guinea pig until 24 h were allowed to elapse between dextran and SRBC. With a 48 h interval between dextran and SRBC, animals of both species had approximately the same number of DPFC and both demonstrated a dramatic cessation of IgG synthesis (Fig. 3). This indicates that the stimulating factor that provided an increased synthesis of IgG at the early intervals was not only absent by 48 h but that some inhibiting factor caused cessation of IgG production. At the same time, however, IgM production remained stimulated. An inhibitor of IgG is inferred since in animals not treated with dextran, production of IgG is greater than in those that are pretreated with dextran at 48 h. These data suggest that the factor that stimulates IgM may act also to depress IgG and vice versa. Were this so, only two factors would be required to explain both increases and decreases in synthesis of these two immunoglobulins.

Thus far, one stimulator and one inhibitor have been described for IgM synthesis (39). Working with the mouse spleen cells' response to SRBC, Dutton has shown that Con A inhibits the in vitro response. He describes one short-lived T cell that produces an inhibitor effect upon B cells and a separate T cell that produces a stimulating effect. Whether these same factors would affect IgG synthesis is as yet unknown.

Resolution of the problems of one vs. two sets of cells for IgM and IgG synthesis as well as how many factors are involved in initiation and cessation of immunoglobulin synthesis must await results of further work which may well be benefited by the use of dextran.

In view of the fact that such drastic alterations in immunological responses can be induced by administering dextran, its clinical uses may well bear closer scrutiny. We are persuaded to this view by initial observations of mice given Maloney sarcoma virus and treated with dextran. Depending upon when it was given relative to the virus, dextran caused threefold increases in tumor size over those of controls (Battisto and Pappas, unpublished data).

SUMMARY

Dextran, of the variety commonly used as plasma expander, markedly altered antibody synthesis to an unrelated antigenic stimulus, SRBC, in two animal species, guinea pig and mouse. The time at which dextran was administered relative to antigen was found to be most critical for increasing or decreasing the number of IgM and IgG PFC. Furthermore, these times differed for the two species studied.

Typically, when given to guinea pigs 6 h before SRBC, dextran caused a 20-fold rise in IgM-producing cells but had little effect upon IgG synthesis. However, if dextran preceded antigen by 24 h the same magnitude of increase was seen in IgG-forming cells while a decrease in IgM-producing cells occurred.

In mice, a short 2 h interval between dextran and antigen favored cells synthesizing IgG and not those producing IgM. A longer 6 to 24 h lapse between dextran and antigen resulted again in an inverted pattern, i.e., an increase in IgM and a decrease in IgG-producing cells.

In both species, if dextran was given 48 h before antigen, synthesis of IgG markedly decreased.

At the cellular level dextran activated those B cells already in the vascular compartment. In stimulating the IgM response to SRBC, dextran appears either to substitute for T cells or to amplify the effects of that small number of T cells still present in bone marrow preparations. Dextran-altered mouse B cells synthesized SRBC-specific IgG in the presence of normal T cells at an earlier time than did normal B and T cells. However, dextran was unable to cause blastogenesis in vitro of guinea pig lymph node cells or mouse B, T, and spleen cells.

The data suggest at least two effects that T cells exert upon B cells. One is to stimulate more B cells to produce IgM, a function accomplished by endotoxins, PWM, PPD, and the simple polysaccharide dextran. The other is to trigger shifts in synthesis of immunoglobulins M and G. Our observations are compatible with the view that a single cell is capable of synthesizing both of these immunoglobulins and that the stimulating factor for one may cause cessation of the other.

Note Added in Proof.—After submission of this article we learned of the work by Diamantstein et al. 1971. *Eur. J. Immunol.* **1**:302; 335; 340; 426; 429. They document in mice the stimulation of humoral antibody formation by the polyanions dextran sulfate, polyacrylic acid, and polymannuronic-guluronic acid. Further, they suggest that polyanions substitute for thymus cell function in the immunological response of bone marrow cells to sheep erythrocytes.

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