ANTIBODY SYNTHESIS AT THE CELLULAR LEVEL

Antibody-Induced Suppression of 19S and 7S Antibody Response

By GÖRAN MÖLLER, M.D., and HANS WIGZELL

(From the Department of Tumor Biology, Karolinska Institutet Medical School, Stockholm, Sweden)

(Received for publication, February 9, 1965)

Specific inhibition of the immune response has been achieved by two different procedures, acting presumably by different mechanisms. Acquired immunological tolerance or immunological paralysis is induced in certain experimental situations by the introduction of antigen. A similar non-responsiveness may result from the passive transfer of specific antibodies prior to antigen injection. Although there are certain similarities between tolerance and antibody-induced inhibition of the immune response, such as the requirement for the continuous presence of the inductive agent (antigen and antibody, respectively) they are clearly distinguished by fundamental differences in the status of the immunological system in the treated hosts. Transfer of lymphoid cells from tolerant animals to immunologically incompetent recipients does not lead to an immediate regain of immunological reactivity against the specific antigens (for discussion see reference 1), whereas similar passage of cells from antibody-treated to untreated hosts results in normal antibody synthesis (2) upon subsequent injection of the corresponding antigen.

Inhibition of the humoral component of the immune response by passively administered antibodies has been demonstrated in a variety of experimental systems using particulate antigens, such as viruses, bacteria, red cells and nucleated tissue cells, and soluble antigens, such as bacterial toxins as well (for references, see reference 3). In isoantigenic systems of the H-2 type in mice, both the humoral and the cell-bound immune response could be inhibited in this way, leading to dramatic changes of the transplantation behavior of normal and neoplastic tissues (immunological enhancement) (4, 5).

The antibody-mediated inhibition of the immune response may reveal the existence of a feed-back system regulating the level of antibody synthesis with regard to a given antigen. Several hypotheses have been put forward concerning the mechanism involved (6–10). According to one of them antibodies inhibit the immunological capacity of the antibody-producing cells specifically and directly (central inhibition of the immune response). This hypothesis was based on the fact that efficient inhibition could be obtained during the first 3 to 6 days after the antigen (8). However, other findings suggested that the antibodies acted primarily at the antigenic level (afferent inhibition)

by combining with the antigenic determinants and thus resulted in the suppression of the induction of the immune response (2, 9, 11). In transplantation system this mechanism of antibody action resulted in efferent inhibition of the homograft reaction in addition, since antibody-coated cells were protected from destruction by immune lymphoid cells (2, 9).

The present investigation represents an attempt to study the mechanism(s) by which passively transferred antibodies suppress antibody production. The Jerne method was applied (12) to study the antibody production of individual cells against sheep red cells *in vitro*. Particular interest was focused on the effect of passively transferred antibodies given at various intervals after antigen injection. The inhibiting capacity of purified 19S and 7S antibodies has been investigated separately. Also, the effect of passively transferred antibodies on 19S and 7S production was examined, in an attempt to clarify some of the factors involved in the regulation of antibody synthesis.

Materials and Methods

Mice of the following inbred strains were used; A/SnKl, A.CA/Kl, A.BY/Kl, A.SW/Kl, C3H/Kl, C57BL/Kl, C57L/Kl, and CBA/Kl. Some experiments were performed with F_1 hybrids of two of these strains. Within each experiment all mice were of the same sex and age. The mice were bred and kept in accordance with procedures worked out in our laboratory (13).

Antibodies were produced by intraperitoneal injections of 4×10^8 sheep red cells. The animals were bled at various intervals depending on the type of antibody required. When high titers of 19S antibodies were needed, they were bled after 5 days; *i.e.*, the time of maximal 19S production in this system. 7S antibodies were derived from hyperimmune sera collected after at least 2 but usually up to 7 weekly intraperitoneal injections of 4×10^8 sheep red cells; the mice were bled 5 to 6 days after the last injection. However, all antisera were separated into 19S and 7S components by sephadex G-200 gel filtration before they were used in the experiments.

Serological Procedures.—Agglutination was performed in the presence of polyvinylpyrrolidone (PVP) and hemolysis in the presence of guinea pig serum as a source of complement (13).

Antibody Fractionation into 7S and 19S components was performed by sephadex G-200 gel filtration (13).

The Agar Plaque Technique for detection of individual antibody-producing cells *in vitro* was performed according to the method of Jerne (12).

Inhibition Experiments.—Each mouse was given 0.1 ml of a sheep red cell suspension containing 4×10^8 cells intraperitoneally or intravenously. 0.1 ml mouse anti-sheep red cell serum was injected 2 hours to 15 minutes before the red cells or 1, 2, 3, or 4 days later.

In experiments designed to test the inhibiting capacity of purified 19S and 7S antibodies, analogous procedures were used and fractions isolated from 0.1 to 0.3 ml whole serum were injected into each recipient prior to the red cells. In some of these experiments 20 times fewer red cells (2×10^7) were injected to each recipient and the antibody fractions were obtained from 1 to 2 ml serum.

RESULTS

Antibody-Induced Inhibition of the Plaque-Forming Ability of Spleen Cells.—Plaque-forming cells (PFC) in mouse spleens started to increase in number

about 24 hours after the injection of sheep red cells, and rose exponentially during the subsequent 3 to 6 days, depending on the mouse strain and the antigen dose (see reference 13). Thereafter, the number of PFC decreased rapidly, but significant numbers were still present after 18 days (for a typical example, see Fig. 3). The increase in the number of plaque-forming cells was paralleled by the increase of the hemolytically efficient, 2-mercaptoethanol(2-ME) sensitive, 19S antibodies, whereas the decrease in the number of PFC, occurring 4 to 5 days after injection of the antigen coincided in time with the appearance of 7S, 2-ME resistant antibodies, detectable by agglutination and, to a lesser degree also by hemolysis. The hemolytic plaque technique thus offered possibilities to study the effect of passively transferred antibodies on individual lymphoid cells producing 19S antibodies.

As a first test the effect of passively transferred antibodies given prior to the antigen was studied. Several such experiments were performed in various mouse strains. 0.05 to 0.2 ml hyperimmune mouse anti-sheep red cell serum of different titers were injected 15 minutes to 2 hours before the injection of 4×10^8 sheep red cells. In some experiments the sheep red cells were treated with high titered antiserum *in vitro* and washed several times prior to injection. Similar results were obtained with both procedures and they will be reported together.

It was regularly found that passively transferred anti-sheep red cell antibodies prior to the injection of sheep red cells caused a pronounced inhibition of the number of PFC during the entire test period (8 days) (Table I). Usually the number of PFC was the same during the entire period as in the non-injected controls (0 to 100 plaques per spleen), but occasionally even lower numbers were found. Recipients not exposed to antibody formed 3.000 to 200.000 plaques per spleen 4 to 5 days after antigen injection.

In some experiments with weak sera the inhibition was not complete. It was usually found that the suppression of the number of plaque-forming cells decreased with lower antibody doses (Table I, experiments 5 and 6) (Fig. 1). This was revealed by a lower maximum number of PFC at days 4 and 5. In some experiments very low antibody doses (0.01 to 1 μl) caused an increase in the number of PFC compared to the non-antibody–treated controls. One such experiment is illustrated in Fig. 1. At present it is not known what the conditions are for this effect to occur.

Number of PFC after Passive Antibody Transfer Following Antigen Administration.—The demonstration that antibodies injected prior to the antigen, or directly absorbed onto the sheep red cells, were capable of depressing the number of antibody-producing cells is in agreement with previous findings in other experimental systems (2, 11). Various experiments outlined in the introduction led to the conclusion that antibodies inhibited the immune response primarily by combining with the antigenic determinants. Other findings obtained by Brent and Medawar (8) suggested, however, that antibodies inhibited antibody formation directly at the cellular level, since they were capable of acting after

TABLE I

Inhibition of the Plaque-Forming Ability of Spleen Cells by Humoral Antibodies Given Prior to the Antigen Injection

Exp. No.	Mouse strain	No. of mice per determination	No. of sheep red cells injected	Antiserum treatment		Mean No. and range of plaque-forming cells per spleen at the following days after antigen injection							Mean No. and range of PFC per 10^6 spleen cells at the following days after antigen injection			
				Vol.	Titer*	2	3	4	5	6	7	8	2	3	4	5
				ml												
1	(A × C3H)F_1	2	4×10^8	—	—	210	2183	19650	8125				2.0	12.6	98.8	65.7
						205–215	2090–2275	18400–20900	7200–9050				1.8–2.1	12.3–12.8	98.6–98.9	56.7–64.6
	"	2	"	0.1	1/8000	7.5	263	273	223				0.08	1.8	1.2	1.7
						5–10	205–320	270–275	215–230				0.07–0.09	1.5–2.1	1.0–1.4	1.2–1.1
	"	1–2	—	—	—	2.5	5	10	30				0.04	0.04	0.07	0.2
						0–5							0.0–0.07			
2	A.CA	2	4×10^8	—	—	150	5150	13425	19588				1.5	50.6	164.3	
						65–235	4600–5700	12700–14150	18675–20500				0.8–2.2	48.4–52.8	147.8–181.2	
	"	2	"	0.1	1/1000	20	175	130	495				0.4	2.5	1.2	
						20–20	155–195	65–195	430–560				0.3–0.4	2.0–2.9	0.8–3.4	
	"	1–2	—	—	—	6	10	0	10				0.09	0.11	0.00	
						5–6							0.08–0.09			
3	A.BY	2	4×10^8	—	—	225	1218	4525	7813		1210	160	5.3	16.8	66.2	
						200–250	835–1600	3400–5680	7250–8375		1020–1400	150–170	4.9–5.6	13.3–20.3	60.7–71.6	
	"	2	"	0.2	1/2000	0	18	3	50		3	0	0.0	0.5	0.03	
						0–0	15–20	0–5	5–95		0–5	0–0	0.0–0.0	0.4–0.6	0.0–0.05	
	"	1	—	—	—	5	20	5	5		5	2	0.06	0.3	0.08	
4	(A × C3H)F_1	2	4×10^8	—	—		2225	32050	11525		3600			12.5		
							2050–2400	31350–32750	8500–14550		2895–4325			8.6–16.9		
	"	2	"	0.1	1/8000		50	63	45		101			0.35		
							50–50	50–75	40–50		13–188			0.34–0.36		
	"	1	—	—	—		30	10	0					0.14		

5	A.CA	3	4×10^8	—	—			9500								57.3	
								3800–16500								21.0–107.0	
	"	3	"	0.05	1/100000			0								0	
								0–0								0–0	
	"	3	"	0.05	1/10000			633								4.9	
								400–950								3.0–6.2	
	"	2	"	0.05	1/1000			1200								3.1	
								1100–1300								6.8–7.3	
	"	3	"	0.05	1/100			26133								190	
								11400–45000								57–304	
6‡	(A × CBA)F_1	4	4×10^8	—	—			72750								653	
								41000–105000								494–921	
	"	3	"	0.025	1/50000			130								1.22	
								20–300								0.21–2.50	
	"	4	"	0.025	1/5000			1150								10.3	
								700–1500								8.1–12.9	
	"	4	"	0.025	1/500			27500								271	
								17000–37000								173–402	
7	(A × CBA)F_1	4	4×10^8	—	—			116000								666	
								57000–214000								277–1029	
	"	4	"	0.025	1/50000			2850								22	
								1800–4000								13–28	
8	CBA	4	4×10^8	—	—			70750								624	
								35000–127000								421–992	
	"	4	"	0.1	1/243			5775								40.5	
								2500–8500								7.5–91.3	
9	(A × C3H)F_1	3	4×10^8					13800								65.0	
								10000–20900								53.6–87.1	
	"	3	"	0.1	1/512			76.7								0.54	
								25–105								0.21–0.93	
	"	1		—	—			5								0.08	

* The titers refer to hemagglutination of sheep red cells in the presence of PVP.

‡ In contrast to all other experiments the red cells were treated with antibodies *in vitro* and washed prior to their inoculation.

Table I, continued

TABLE I—*Concluded*

Exp. No.	Mouse strain	No. of mice per determination	No. of sheep red cells injected	Antiserum treatment		Mean No. and range of plaque-forming cells per spleen at the following days after antigen injection							Mean No. and range of PFC per 10^6 spleen cells at the following days after antigen injection			
				Vol.	Titer*	2	3	4	5	6	7	8	2	3	4	5
				ml												
10	A.SW	4	2×10^7	—	—			1998 900–4880								
	"	4	"	0.2	1/256			7.5 0–20								
	"	1	—	—	—			30								
11	A.SW	3	2×10^7	—	—			1650 1800–2300								
	"	4	"	0.1	1/2000			8.3 0–23								
	"	4		—	—			17.5 5–35								
12	(A × C3H)F_1	4	4×10^8					32500 20000–55000							198.6 125.0–268.2	
	"	4	"	0.1	1/64			2513 1000–4150							21.5 6.6–29.5	
	"	1		—	—			0							0	
13	(A × C3H)F_1	4	2×10^7	—	—			3243 1025–6540								
	"	4	"	0.1	1/64			265 140–350								
	"	1	—	—	—			20								

the homograft reaction had been already initiated. This problem was now reinvestigated by the hemolytic plaque technique, which permits a study at the cellular level.

As a first step, antibodies were transferred passively 1, 2, 3, and 4 days after the intravenous administration of sheep cells and the number of PFC was determined on day 4. The antiserum-treated groups received 0.1 ml of a hyperimmune mouse anti-sheep red cell serum (agglutinin titer 3^8). A control group received the antigen only. On day 4 (3 hours after the last antiserum injection) the mice were killed and the number of PFC determined.

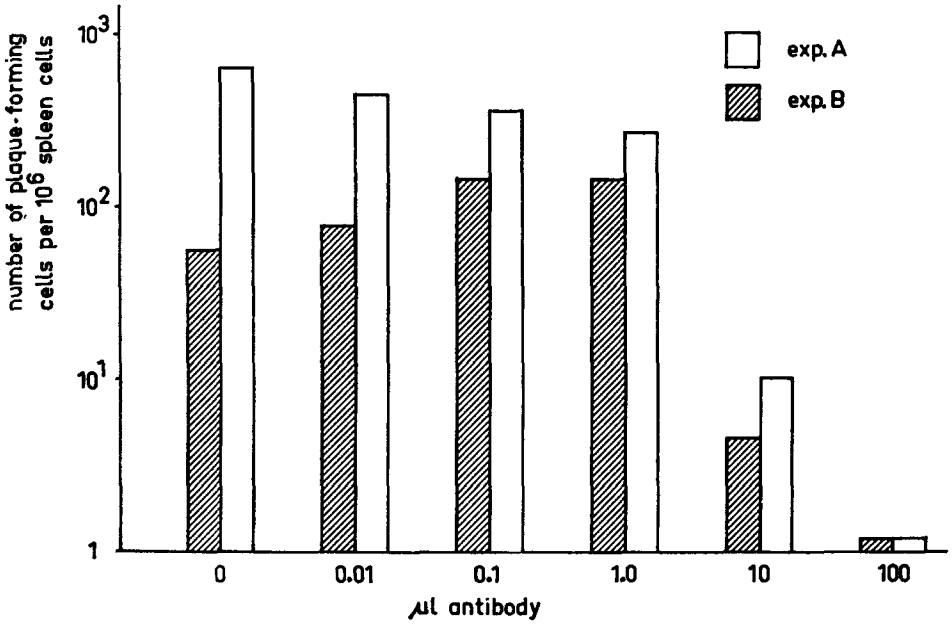


FIG. 1. Effect of various doses of mouse anti-sheep red cell serum injected at the same day as the antigen on the number of plaque-forming spleen cells 4 days later in (A × CBA) F_1 mice (experiment A) and in A.CA mice (experiment B). Each bar represents the mean value of 4 mice.

As illustrated in Fig. 2 there was a marked decrease in the number of PFC if antibodies were given 1 and 2 days after the antigen. Antiserum given 3 and 4 days after the antigen (less than 40 hours before testing) had a negligible effect only.

A more detailed study with a similar purpose was subsequently undertaken by injecting groups of 8 to 20 mice with sheep red cells followed by antibodies at various intervals. Starting the 2nd day after antigen injection, 2 non-antibody-injected controls and 2 antibody-treated mice of each group were killed and the number of PFC determined. Different experiments performed with

different mouse strains gave similar results, two of which are illustrated in Figs. 3 *a* and 3 *b*. Antibodies given 1 hour after the antigen caused a complete suppression of PFC. The effect of passively transferred antibodies given after 1 to 4

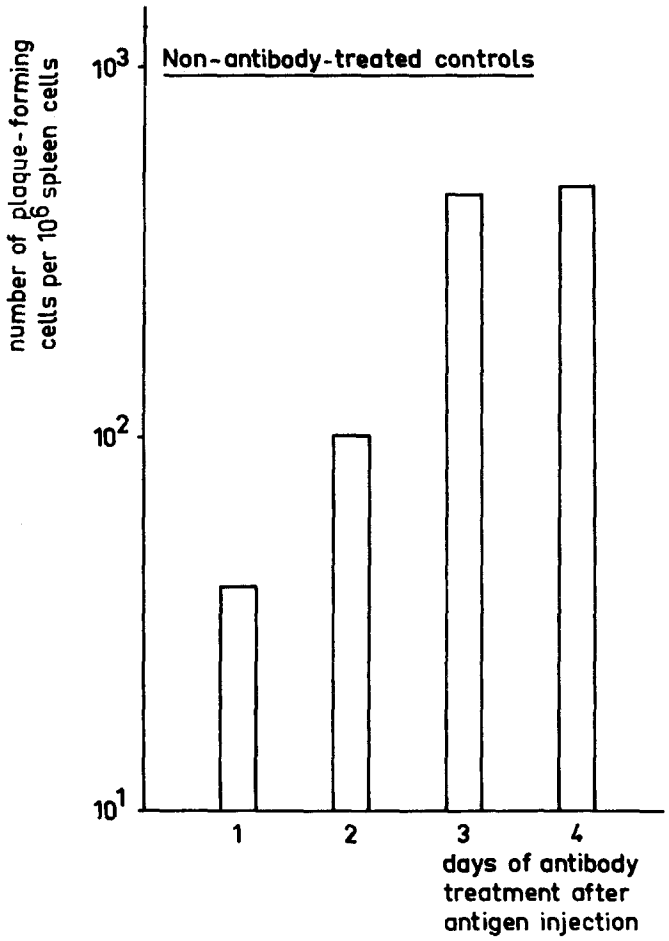
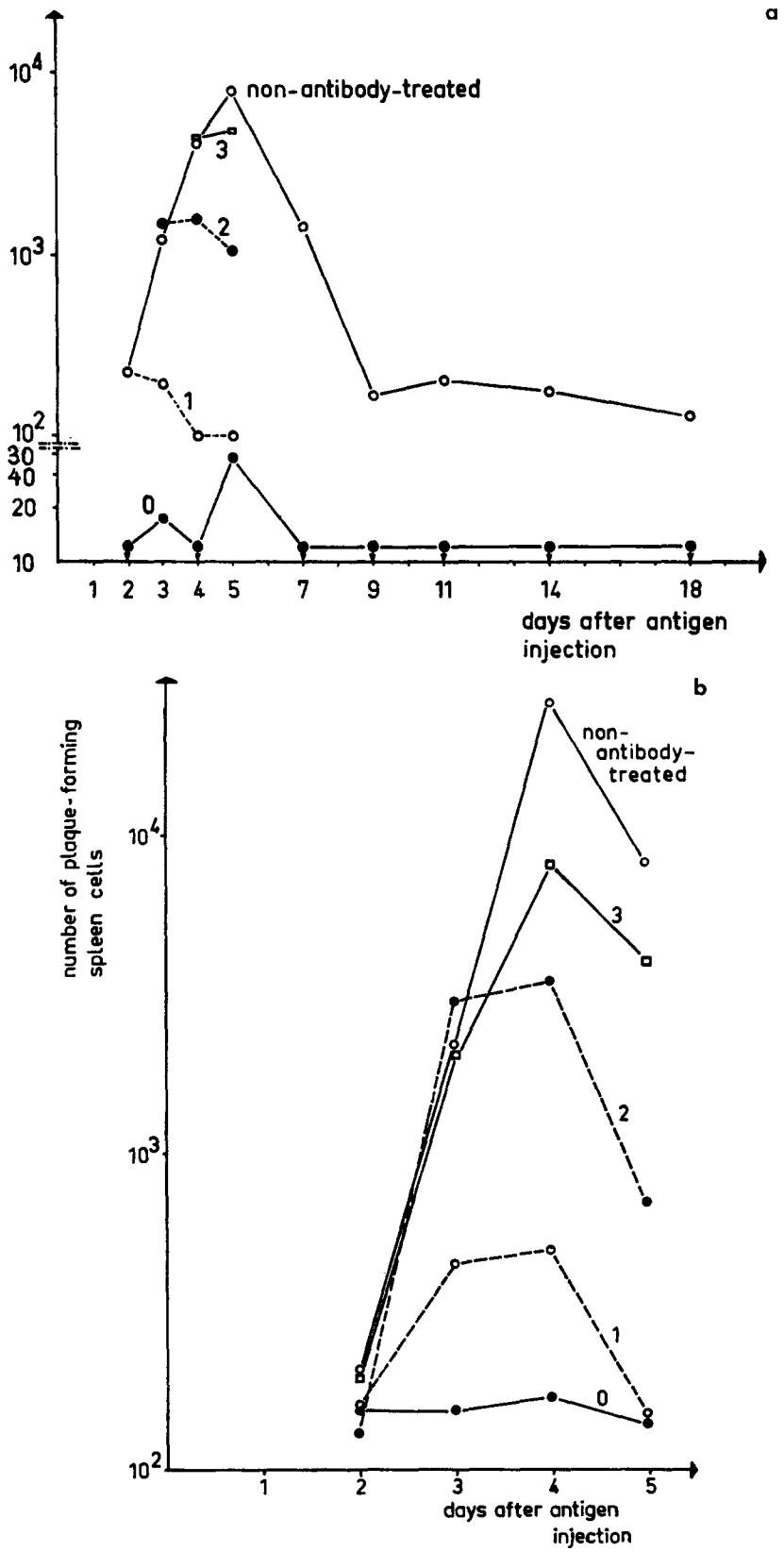


FIG. 2. Number of PFC in CBA mice 4 days after the injection of sheep red cells. One group remained untreated, whereas the others were given mouse anti-sheep red cell serum at the indicated days. Each bar represents the mean value of 4 mice.

days was manifested after a latency period of 48 hours as the termination of the exponential increase in PFC numbers. In no case did the injected antibodies detectably inhibit the increase of PFC numbers during the first 24 hours. The interval of 48 hours before the observable effect of passively transferred antibodies was the same whether the antibodies were administered 1, 2, or 3, days



FIGS. 3 *a* and 3 *b*. Development of plaque-forming spleen cells in A.BY mice (Fig. 3 *a*) and (A × C3H)F_1 hybrids (Fig. 3 *b*) after injection with sheep red cells alone or followed by mouse anti-sheep serum at the indicated days. Each point represents the mean value of 2 mice.

after the antigen. Subsequent to the end of the exponential phase the number of PFC in the antibody-treated animals decreased at the same rate as the non-antibody–treated controls after the peak day.

A few experiments were performed to study more precisely the time needed for passively transferred antibodies to inhibit the appearance of the PFC. A typical experiment is illustrated in Fig. 4, where anti-sheep red cell antibodies were injected 24 hours after the antigen and the number of plaque-forming cells was

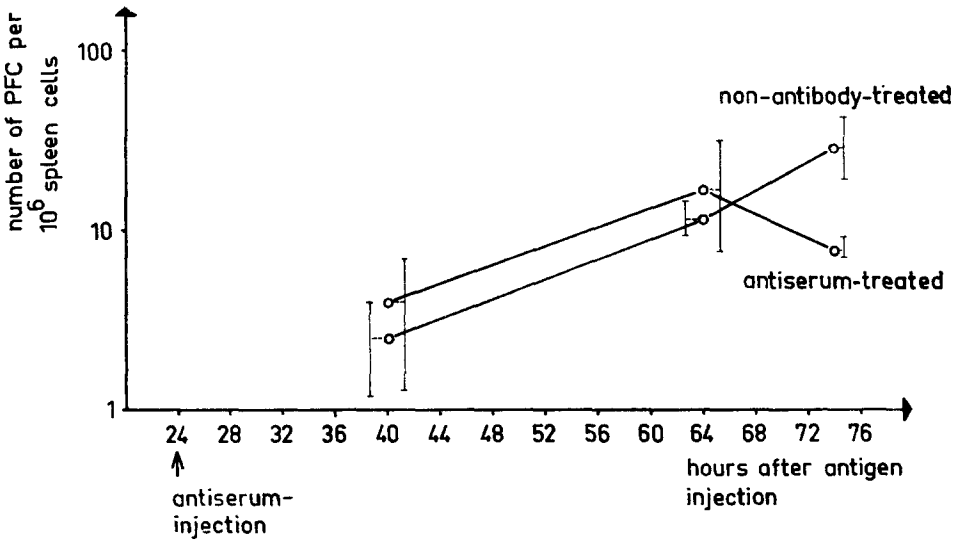


FIG. 4. Development of plaque-forming spleen cells in A.BY mice at various times after the injection of sheep red cells alone or sheep red cells followed by antiserum 24 hours later. Each point represents the mean value of 4 mice. The range is indicated by vertical bars.

determined at 16, 40, and 50 hours thereafter. Spleen cells removed after 16 and 40 hours contained the same number of PFC as the non-antibody–treated controls, whereas a pronounced difference was found after 50 hours.

The doubling time of PFC numbers during the exponential phase varied between 5 and 12 hours in different experiments. On basis of the hypothesis (13) that cellular proliferation is mainly responsible for this increase it follows that 3 to 8 cell divisions occurred in the presence of antibodies without any detectable inhibition. It would follow that antibodies did not inhibit the antibody synthesis of already committed cells directly. It seems more likely that they counteracted the stimulus inducing the cell divisions responsible for the increase in the number of PFC, although this was not manifested during the first 40 hours.

Inhibiting Effect of 19S and 7S Antibodies.—In the experiments described above, whole serum was used as a source of antibodies. It is of importance to

determine what type of antibody is responsible for the inhibition of PFC formation. As a first step the effect of purified 19S and 7S antibodies was tested. The antibody fractions were prepared by sephadex G-200 gel filtration (13). The 19S fractions were tested for possible contamination with 7S antibodies by treating samples of the pooled and concentrated fractions with 2-ME and thereafter testing for residual agglutinin activity. In no cases were 2-ME resistant 7S agglutinins detected in the 19S fractions.

Concentrated fractions derived from 0.1 to 0.2 ml mouse anti-sheep red cell sera were injected intraperitoneally 30 minutes prior to the injection of the sheep red cells. Control groups received sheep red cells only. Four days later the spleens of the recipients were tested by the Jerne method. Four experiments of this design were performed in different mouse strains and they all had similar results.

As can be seen from Table II (experiments 1 to 4) 7S antibodies caused a pronounced suppression of the number of PFC, whereas 19S antibodies were much less efficient. The source of the antibodies did not appear to influence the results, since the findings were the same whether the 19S antibodies were isolated from hyperimmune sera or from the sera of mice given a first antigen injection 5 days previously. Even in cases (experiment 3) where the agglutinin titers of the 19S and 7S fractions were the same and the hemolytic titers of 19S therefore considerably higher, only the 7S antibodies inhibited the immune response.

It seemed possible, however, that this difference between 19S and 7S antibodies was quantitative rather than qualitative. It has been reported that the agglutinating and hemolytic efficiency of 19S antibodies is relatively higher than their precipitating activity, whereas this was not the case with 7S antibodies (14).

In an attempt to detect a possible suppressing effect of 19S antibodies on the immune response, analogous experiments were performed, but the antibody fractions injected were derived from sera with higher titers and larger volume (0.25 to 1 ml) were used per recipient (experiments 5 and 6, Table II). The sheep red cell dose was decreased from 4×10^8 to 2×10^7 (experiments 7 to 11). This means a 100- to 200-fold increase of the antibody/antigen ratio. Several experiments of this type were performed as described previously (Table II, experiments 5 to 11) (Fig. 5). As shown in Table II, 19S antibodies were capable of inhibiting the appearance of PFC. The degree of inhibition varied in different experiments and was usually somewhat smaller than with 7S. However, in experiments 7, 8, and 9, 19S and 7S inhibited to approximately the same degree. In experiment 9 the serum was taken 5 days after the first antigen injection, when 19S antibodies had reached their maximum value, whereas only small amounts of 7S were produced. This experiment makes it very unlikely that 7S contamination of the 19S fraction was responsible for the inhibition, since the purified fractions were of equal efficiency.

TABLE II

Inhibition of the Plaque-Forming Ability of Spleen Cells by Purified 19S and 7S Antibodies Given Prior to the Antigen Injection

Exp. No.	Mouse strain	No. of mice	No. of sheep red cells	Antiserum			Mean No. and range of PFC per spleen at 4 days	Mean No. and range of PFC per 10^6 spleen cells at 4 days
				Vol.	Fraction	Titers*		
1	(A × C3H)F_1	4	4×10^8	—	—	—	32500 20000–55000	197.7 125.6–254.6
	"	4	"	0.1	19S	0‡ 1/64‡	21875 11000–37500	237.0 125.0–468.8
	"	4	"	0.1	7S	1/32‡ 1/16‡	8363 3300–16000	37.2 29.5–65.8
	"	1	—	—	—	—	0	0
2	(A × C3H)F_1	3	4×10^8	—	—	—	13733 10000–20700	
	"	3	"	0.1	19S	1/8 1/256	10617 4350–13800	100.4 49.4–137.0
	"	3	"	0.1	7S	1/512 1/32	76.7 25–105	0.54 0.21–0.93
	"	1	—	—	—	—	5	0.08
3	(A × C3H)F_1	3	4×10^8	—	—	—	38700 33100–43200	203.3 191.3–213.1
	"	3	"	0.2	19S	1/8 1/5000	20633 14700–25200	175.2 142.9–210.0
	"	3	4×10^8	0.2	7S	1/8 1/4	383 275–440	2.9 2.4–3.4
	"	1	—	—	—	—	88	1.3
4	A.BY	3	4×10^8	—	—	—	3395 1935–5250	
	"	3	"	0.1	19S	1/128 1/512	3482 3145–3750	
	"	1	—	—	—	—	45	

* The first titer refers to agglutination, the second to hemolysis in the presence of guinea pig complement. In experiments 10 and 11 only hemolytic titers were determined. Unless indicated otherwise the titers refer to the peak 19S and 7S fractions after sephadex gel filtration.

‡ Titers of pooled and concentrated 19S and 7S fractions.

TABLE II—*Continued*

Exp. No.	Mouse strain	No. of mice	No. of sheep red cells	Antiserum			Mean No. and range of PFC per spleen at 4 days	Mean No. and range of PFC per 10^6 spleen cells at 4 days
				Vol.	Fraction	Titers*		
5	(A × A.CA)F_1	3	4×10^8	—	—	—	2967 1150–4050	36.1 30.5–44.0
	"	3	"	0.3	19S	1/160 1/2500	765 540–1040	9.7 7.8–10.4
	"	3	"	0.3	7S	1/2500 1/10	392 290–500	4.2 2.8–6.3
6	A.CA	3	4×10^8	—	—	—	34900 15700–47700	
	"	3	"	1.0	19S	1/128 1/1024	12767 9400–16250	
	"	1	—	—	—	—	60	
7	A.SW	4	2×10^7	—	—	—	1999 900–4880	
	"	4	"	0.25	19S	1/64 1/256	41 15–50	
	"	4	"	0.25	7S	1/256 1/64	2.5 0–20	
	"	1	—	—	—	—	30	
8	A.SW	3	2×10^7	—	—	—	1650 1150–2300	
	"	4	"	0.5	19S	1/16 1/256	33.2 10–58	
	"	4	"	0.5	7S	1/5000 1/32	5.0 0–20	
	"	4	—	—	—	—	16.5 5–35	
9	(A × C3H)F_1	4	2×10^7	—	—	—	3568 1025–6540	
	"	3	"	1.0	19S	1/32‡ 1/5000‡	286.7 10–450	
	"	4	"	1.0	7S	1/16‡ 1/16‡	285.0 150–420	

TABLE II—*Concluded*

Exp. No.	Mouse strain	No. of mice	No. of sheep red cells	Antiserum			Mean No. and range of PFC per spleen at 4 days	Mean No. and range of PFC per 10^6 spleen cells at 4 days
				Vol.	Fraction	Titers*		
9, con't	(A × C3H)F_1	1	—	—	—	—	20	
10	A.SW	4	2×10^7	—	—	—		125.0 51.0–179.0
	"	2	"		19S	1/256‡		0.24 0.23–0.24
	"	3	"		"	1/128‡		1.8 0.78–3.75
	"	3	"		"	1/64‡		25.3 11.9–42.0
	"	3	"		7S	1/16‡		0.0 0–0.1
	"	3	"		"	1/8‡		0.4 0–1.3
	"	3	"		"	1/4‡		0.9 0.2–2.0
	"	3	"		"	1/2‡		2.3 0.45–6.5
11	A.SW	3	2×10^7	—	—	—		49.0 32.0–75.0
	"	3	"	0.5	19S	1/81‡		16.2 1.6–31.0
	"	3	"	0.5	7S	1/3‡		3.6 1.6–6.7

The inhibiting effect of various doses of 19S and 7S antibodies was studied quantitatively. As illustrated in Fig. 5 the degree of inhibition decreased with diminishing antibody doses; in the case of the higher dilutions of 19S, 1/25 and 1/125 became actually stimulating, as compared to non-antibody–treated controls. A comparison between the hemolytic serum titers and the degree of PFC suppression indicated that 7S antibodies were about 115 times more efficient than 19S antibodies at all dilutions.

Thus, it would appear that both 19S and 7S antibodies are both competent to inhibit the appearance of antibody-producing cells, although there is a marked difference in the relative efficiency of the two antibody types, 7S being about 100 to 200 times more efficient when compared on the basis of the same hemolytic titers.

Antibody-Induced Inhibition of the 7S Response.—The previous studies have been concerned with antibody-induced depression of the PFC, as detected by the Jerne technique. As shown (13) the plaque-forming ability is presumably related to 19S antibody production whereas the marked increase in 2-ME

resistant 7S antibodies occurring after 5 to 7 days is not manifested in an increase of PFC. On the contrary the PFC decrease coincided with an increase of 7S production. Since passively transferred 7S antibodies had a marked ca-

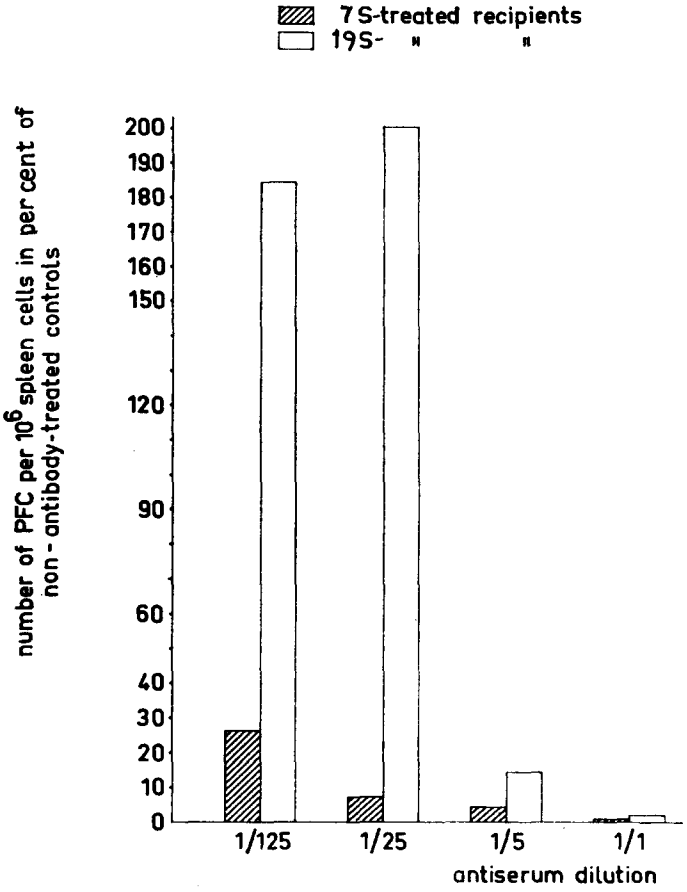


FIG. 5. Effect of various doses 19S and 7S antibodies in A.SW mice given at the same day as the antigen on the number of plaque-forming cells per 10^6 spleen cells 4 days later, as related to the number found in non-antibody-treated recipients. Each bar represents the mean value of 3 mice.

pacity to inhibit 19S synthesis, it seemed possible that actively produced 7S would also inhibit 19S formation. It is noteworthy, however, that 7S synthesis did not appear to be self-inhibiting immediately, since 7S titers continued to increase during the first 12 to 20 days. As a first attempt to analyze the possible absence of such a 7S self-inhibition, it was investigated whether passively transferred antibodies can inhibit 7S production in a manner analogous to the previously studied 19S synthesis.

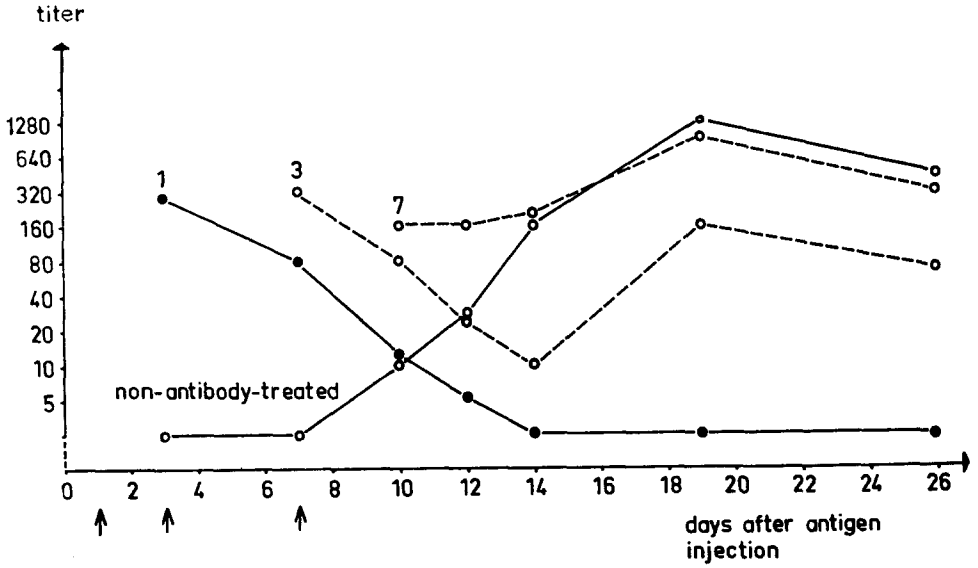


FIG. 6 *a*

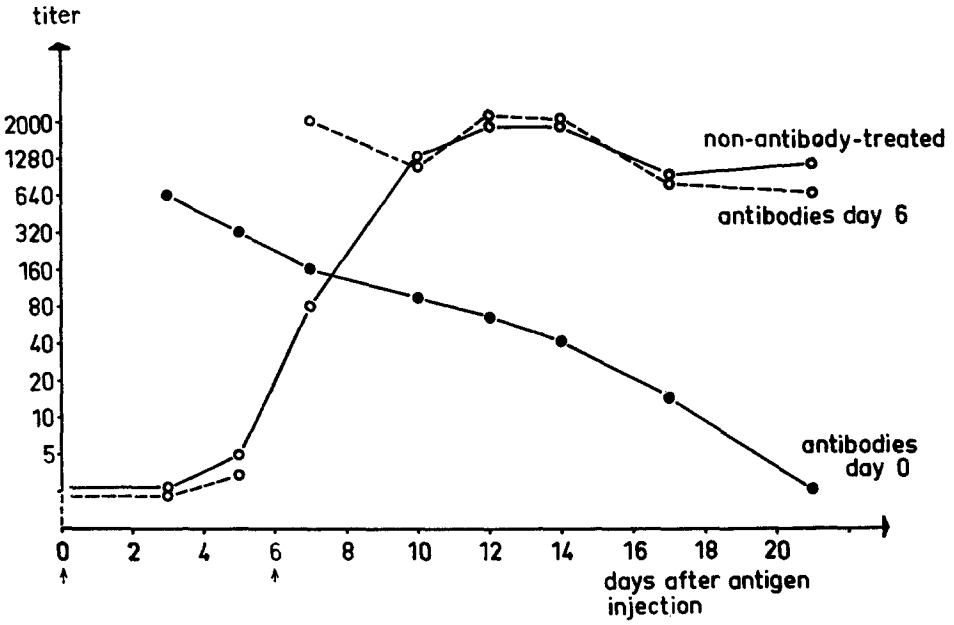


FIG. 6 *b*

FIGS. 6 *a* and 6 *b*. Production of 2-ME resistant 7S anti-sheep agglutinins in A.SW mice (Fig. 6 *a*) and A mice (Fig. 6 *b*) injected with sheep red cells alone or with red cells followed by antiserum at various intervals. The arrows indicate the time of antiserum injection. Each point represents the geometric mean titer of 3 mice.

FIG. 6 *a*. 7S agglutinin titers after injection of sheep red cells alone and red cells followed by antiserum at the indicated days.

FIG. 6 *b*. 7S titers after injection of sheep red cells alone and red cells followed by antiserum at the indicated days.

0.1 to 0.3 ml hyperimmune mouse anti-sheep red cell serum was injected 30 minutes prior to the intraperitoneal injection of 4×10^8 sheep red cells. A control group received red cells only. At various intervals the number of PFC and the production of hemolytic antibodies was studied as in the previous experiments, and, in addition, the titers of agglutinating antibodies were determined before and after 2-ME treatment. Antibody treatment of the recipients resulted in complete suppression of PFC in accordance with the previous results, indicating that there was an efficient inhibition of 19S production. The results reported below will be concerned only with the effect of passively transferred antibodies on the synthesis of 2-ME resistant 7S agglutinins.

As illustrated in Figs. 6 *a* and 6 *b* active production of 7S agglutinins was completely suppressed by passively transferred antibodies given before the antigen, and only the decay of the administered antibodies was observed. Suppression was complete during the entire test period (27 days). This is in agreement with previous results obtained with bacterial antigens and cellular isoantigens of the H-2 system in mice (2, 3, 5).

Further studies indicated that passive transfer of antibodies within the first 24 hours after injection of sheep red cells equally well suppressed 7S antibody production (Figs. 6 *a* and 6 *b*) in analogy with the results on 19S antibody systhesis. Twenty-four hours after injection the sheep red cells have localized intracellularly in the spleen and liver, as shown by studies with Cr^{51}- labeled cells (13), and 19S synthesis has been initiated.

As a further step it was investigated whether antibodies given at various intervals after the antigen would suppress 7S antibody synthesis. Several different experiments were performed. In most cases simultaneous determinations of PFC, hemolytic antibody titers, and agglutinating titers before and after 2-ME treatment were determined, but in some only agglutinins were tested after treatment with 2-ME.

It was found that antibodies given at the 3rd day after the antigen injection caused a delayed production of 7S agglutinins (Figs. 6 *a* and 6 *b*). However, antibodies given at the 5th day or later, at the beginning of detectable 7S antibody production, did not decrease the subsequent active production of 7S agglutinins and the titers in this group were identical to those obtained in non-antibody–treated controls (Figs. 6 *a* and 6 *b*).

DISCUSSION

As reported above, passively transferred anti-sheep red cell antibodies inhibited completely the development of the PFC if given before the antigen. Antibody-induced suppression of the appearance of PFC was achieved also after antibody synthesis had been initiated, although a detectable effect was not observed before 40 hours after the antibody transfer. The mechanism by which antibodies suppress the immune response is not known as yet. Similar studies in transplantation systems involving the H-2 system of the mouse led to the conclusion that the antibodies acted primarily by interfering with the antigenic

determinants and not by suppressing directly the antibody production of the immunologically competent cells. The present findings substantiate this conclusion by demonstrating that passively transferred antibodies did not directly inhibit the antibody synthesis of already producing cells. There is at present little evidence to exclude the possibility that passively administered antibodies inhibit the immune response by interacting with the antigenic determinants (afferent inhibition). If so, it follows from the present findings that this interaction can also occur with intracellularly localized antigens, since antibodies were efficient during the first 3 to 4 days after antigen injection as well.

Evidence has been presented by Uhr and Finkelstein (15) suggesting that the presence of antigen is essential for the continuous synthesis of 19S antibodies. Passively transferred antibodies would presumably lead to a functional inactivation of the antigen and would be expected, therefore, to terminate 19S antibody production at any stage after its initiation. Since passive antibody transfer only led to inhibition of 19S antibody synthesis after a latency period of 40 hours, however, it would follow from the above concept that the presence of antigen is not a necessary requisite for antibody production by already committed cells. However, the presence of antigen appears to be essential for the continuous increase in PFC numbers, presumably due to cell divisions. It is not known as yet, whether the sheep red cell antigens are present in the PFC or remain localized in other cell types, such as macrophages, where they are initially taken up. It has been claimed, however, that flagellar antigens of bacteria could not be found in the antibody-producing cells (16). If this is true for sheep red cell antigens as well, it would follow that an antigen localized in some other cell types would be responsible for the stimulation leading to cellular multiplication of the 19S antibody-producing cells. Passively transferred antibodies would interact with the antigen and terminate this stimulation. The fact that inhibition did not become expressed until 3 to 7 cell divisions had occurred could be ascribed to inefficient interaction between the antibodies and the antigen, or alternatively, to a lifetime of approximately 40 hours of the specific stimulation factors.

Although both 7S and 19S antibodies were capable of inhibiting the production of PFC, the efficiency of 7S antibodies was about 100 to 200 times greater when compared at the same hemolytic titers. The difference in efficiency between the two antibody types could not be adequately explained by reference to differences in avidity, which appeared to be of the same order of magnitude for a given serum (17). It seems possible, however, that 19S antibodies were more efficient on a molecular basis compared to 7S antibodies, with regard to serological reactions *in vitro*, such as agglutination and, in particular hemolysis (13). Inhibition of the immune response presumably depends on the saturation of the antigenic determinants (5). Therefore, the number of 19S and 7S antibody molecules attached to the red cells at equal serum titers are dif-

ferent, resulting in unequal degrees of inhibition, since relatively fewer 19S antibody molecules would then be present.

The termination of the 19S antibody production coincided in time with the appearance of 7S antibodies in the serum of the mice. This raised the possibility that 7S antibodies might have inhibited the further development of 19S antibody producing cells. Although alternative explanations do exist, such as a predetermined, limited lifetime of 19S antibody production, or depletion of antigen, the present findings on antibody-mediated inhibition of 19S antibody synthesis are in agreement with the possibility that 7S antibodies inhibit 19S antibody production. This possibility raises in turn the question why 7S antibodies do not inhibit their own production. This cannot be explained by any inherent inability of 7S to suppress the initiation of 7S production, since passive transfer of anti-sheep red cell serum within 24 hours after the antigen injection suppressed completely the production of 2-ME resistant 7S agglutinins. As previously discussed, antibodies did not appear to inhibit antibody synthesis in already committed cells, but seemed to act by removing the stimulus for the cell divisions. The absence of an immediate self-inhibition of 7S production could be satisfactorily explained by the assumption that 7S-producing cells do not divide or divide at a slow rate. It is presently not possible to decide whether 7S-producing cells represent the end product of 19S synthesizing precursor cells, or two cell lines develop in parallel, one responsible for 19S and one for 7S synthesis. Recent studies by Nossal (18) favor the former alternative. The present data would be in agreement with this possibility. According to this hypothesis 19S-producing cells divide and mature, reach a final stage and start to produce 7S antibodies. The latter would inhibit the initiation of new 19S-producing cells through combination with the antigen and therefore suppress the recruitment of their own precursors. This would lead to a steady state of 7S production by cells with a long lifetime. This hypothesis is in agreement with the finding that passively transferred antibodies given 5 to 7 days after the antigen, at a time when 7S production has just started, do not detectably inhibit subsequent 7S synthesis. Antiserum given after 3 days caused a decreased 7S production, as would be expected, since the antiserum caused a slight but detectable decrease in the number of 19S producing cells, thereby inhibiting a fraction of the 7S precursor cells. According to this hypothesis, therefore, 7S antibodies would possess animportant regulatory function and would act in a feed-back system preventing extensive cellular multiplication in response to a single antigen, and, therefore, protect against hyperimmunization and/or excessive multiplication of one category of cells.

SUMMARY

The suppressing activity of passively transferred antibodies on antibody synthesis against sheep red cells was investigated at the cellular level by the

agar-plaque technique developed by Jerne. Humoral antibodies injected prior to the antigen suppressed the appearance of plaque-forming spleen cells producing 19S antibodies completely. Antibodies given during the first 4 days after antigen injection also showed such action, but only after a latency period of 40 hours. The inhibiting efficiency of 7S antibodies was about 100 to 200 times greater than that of 19S antibodies. The results support the conclusion that humoral antibodies inhibit the immune response by removing the stimulus for the proliferation of the antibody producing cells and not by directly depressing antibody synthesis in already committed cells.

Passively transferred antibodies inhibited the 7S response if given prior to, or 24 hours after the antigen injection, in analogy with previous results concerning 19S response. In contrast to these previous results on 19S synthesis, antibody transfer had no detectable effect during the early exponential phase of 7S production (5 to 7 days after antigen injection). Only limited inhibition was observed 3 days after the antigen. One possible explanation of this difference is that 7S-producing cells do not divide, or divide at a slow rate. Antigen injection would stimulate the proliferation of 19S-producing cells. Subsequently these would switch to the synthesis of 7S antibodies. These would inhibit the initiation of new 19S-producing cells by combining with the antigen. They would thus suppress the recruitment of their own precursors. A steady state of 7S antibody production by cells with a long lifetime would be the result. This hypothesis ascribes an important regulatory function to 7S antibodies. They would be parts of a feed-back system preventing excessive cell multiplication in response to a single antigen.

This work was supported by grants from the Swedish Cancer Society, the Swedish Medical Research Council and by grant C-3700 from the National Cancer Institute, United States Public Health Service. The authors wish to thank Miss Lena Lundin and Miss Gertrud Linderoth for skillful technical assistance.

BIBLIOGRAPHY

1. Medawar, P. B., Theories of immunological tolerance, *Ciba Found. Symp. Cellular Aspects Immunity*, 1960, 134.
2. Möller, G., Antibody-induced depression of the immune response: A study of the mechanism in various immunological systems, *Transplantation Bull.*, 1964, **2**, 405.
3. Möller, G., On the role of isoimmune reactions in tissue transplantation, Stockholm, Balders tryckeri, 1963.
4. Snell, G. D., Winn, H. J., Stimpfling, J. A., and Parker, S. J., Depression by antibody of the immune response to homografts and its role in immunological enhancement, *J. Exp. Med.*, 1960, **112**, 293.
5. Möller, G., Studies on the mechanism of immunological enhancement of tumor homografts. I. Specificity of immunological enhancement, *J. Nat. Cancer Inst.*, 1963, **30**, 1153.

6. Snell, G. D., The suppression of the enhancing effect in mice by the addition of donor lymph node cells to the tumor inoculum, *Transpl. Bull.*, 1956, **3,** 83.
7. Billingham, R. E., Brent, L., and Medawar, P. B., "Enhancement" in normal homografts with a note on its possible mechanism, *Transpl. Bull.*, 1956, **3,** 84.
8. Brent, L., and Medawar, P. B., Quantitative studies on tissue transplantation immunity. V. The role of antiserum in enhancement and desensitization, *Proc. Roy. Soc. London, Series B*, 1961, **155,** 392.
9. Möller, G., Studies on the mechanism of immunological enhancement of tumor homografts. III. Interaction between humoral antibodies and immune lymphoid cells, *J. Nat. Cancer Inst.*, 1963, **30,** 1205.
10. Finkelstein, M. S., and Uhr, J. W., Specific inhibition of antibody formation by passively administered 19S and 7S antibodies, *Science*, 1964, **146,** 67.
11. Möller, G., Studies on the mechanism of immunological enhancement of tumor homografts. II. Effect of antibodies on various tumor cells, *J. Nat. Cancer Inst.*, 1963, **30,** 1177.
12. Jerne, N. K., and Nordin, A. A., Plaque formation in agar by single antibody-producing cells, *Science*, 1963, **140,** 405.
13. Wigzell, H., Möller, G., and Andersson, B., Antibody synthesis at the cellular level. Kinetics of the primary 19S immune response, to be published.
14. Shulman, S., Hubler, L., and Witebsky, E., Antibody response to immunization by different routes, *Science*, 1964, **145,** 815.
15. Uhr, J. W., and Finkelstein, M. S., Antibody formation. IV. Formation of rapidly and slowly sedimenting antibodies and immunological memory to bacteriophage × 174, *J. Exp. Med.*, 1963, **117,** 457.
16. Nossal, G. J. V., Ada, G. L., and Austin, C. M., Cellular distribution of flagellar antigens labelled with Iodine-131, *Nature*, 1963, **199,** 1259.
17. Andersson, B., unpublished experiments.
18. Nossal, G. J. V., Szenberg, A., Ada, G. L., and Austin, C. A., Single cell studies on 19S antibody production, *J. Exp. Med.*, 1964, **119,** 485.