ANTIBODY SYNTHESIS AT THE CELLULAR LEVEL

Antibody-Induced Suppression of 7S Antibody Synthesis

By HANS WIGZELL, M.B.

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

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Specific inhibition of the humoral component of the immune response by passively administered antibody has been demonstrated in a variety of experimental systems indicating the important role of antibody as a feedback factor during immune response (1–8, and earlier references quoted in reference 1). Both 19S and 7S antibodies have been found to function as inhibitors, 7S as a rule being more efficient than 19S antibodies (6–8). Passively transferred antibody has been found particularly efficient as an inhibitor if administered prior to or during the induction of both 19S and 7S antibody synthesis, whereas an already producing 7S system has been considered more resistant to inhibition than a comparable 19S system (6–8). This difference between the two antibody systems is in agreement with findings that 7S synthesis in most systems is very long-lived compared to 19S production indicating either that the 7S-producing system is not as easily depleted of antigen as is the 19S system or the 7S synthesizing cells are very long-lived even in complete absence of antigen 9–12).

In the present article the concept of the difference between 19S- and 7S-synthesizing systems with regard to sensitivity to antibody-induced suppression has been challenged. Precise studies of 19S and 7S antibody synthesis at a given moment are now possible at the cellular level using the direct (13) and indirect (14–16) hemolytic plaque tests. Using these techniques, particular attention has been focused on the effect of giving antibody well after the primary peaks of 19S and 7S synthesis.

Material and Methods

Animals.—Mice of the following inbred strains were used: A/SnKl, A.BY/Kl, A.SW/Kl, CBA/Kl, C3H/Kl, and C57BL/Kl. Some experiments were performed using F_1-hybrids of two of these strains. The mice were between 2 and 5 months of age when used. Within each experiment all mice were of the same strain, sex, and age.

Antibodies.—Antibodies for inhibition were produced by repeated intraperitoneal injections of 4×10^8 sheep or chicken red blood cells (SRBC or ChRBC) into groups of 40 to 100 mice. The animals received on the average five to twelve 2-wk injections and were bled 5 to 10 days after the last one. Care was taken to ensure that the animals were immunized at least once against the same batch of erythrocytes that was used in the inhibition experiment(s) for induction of the primary response. The antisera for inhibition were produced and used within the same strain.

The antisera used to develop 7S plaques in the indirect hemolytic plaque test (14, 15) were produced in rabbits immunized with mouse 7S antibodies by the following technique. Mouse anti-human serum albumin (HSA) antibodies of 7S type were obtained after gel filtration on Sephadex G-200. The antibodies were absorbed onto HSA-coated acryl plast particles (Bofors-Nobelkrut, Bofors, Sweden) at room temperature for 1 to 2 hr (17). The particles, now coated with HSA–anti-HSA complexes were washed twice and inoculated into rabbits as 1 ml of a 3% suspension. The rabbits were bled 8 to 10 days after the last of four weekly injections. This procedure regularly yielded antisera with optimal developing activity for 7S plaques (13, 14) at dilution 1:100.

Serological Procedures.—Hemagglutination was carried out in the presence of PVP according to Stimpfling (18). Hemolysis was carried out as previously described (19) but using Cr^{51}-labeled target cells measuring the isotope release in a well-type scintillation counter. This increased precision and allowed the use of 10% specific hemolysis to be used as end point in titrations. Indirect hemolysis was carried out by incubating the cells with antiserum as in ordinary hemolysis for 1 hr at 37°C, washing once and adding the rabbit–anti-mouse 7S antiserum diluted 1:100 together with complement. Fractionation of antisera into 19S and 7S fractions was carried out on Sephadex-200 (19). 2-mercaptoethanol (2ME-) inactivation was carried out according to Uhr and Finkelstein (9).

Hemolytic Plaque Tests.—The direct hemolytic plaque test detecting predominantly 19S-producing cells (13, 19) was carried out as described by Jerne et al. (13). The indirect hemolytic plaque test detecting 7S-producing cells (14–16) was carried out according to Dresser and Wortis (15). A high degree of inhibition of 19S plaques was found when using our unabsorbed rabbit–anti-mouse 7S antiserum confirming the findings of others (15). Using our unabsorbed antiserum in two experimental systems where only 19S antibody synthesis could be recorded the average inhibition of 19S plaques in 22 different tests was found to be 80.4% (SE = 4.1%) (20). Thus, in the present tests when unabsorbed rabbit–anti-mouse 7S antiserum was used 20% of the number of plaque-forming cells (PFC) found in the direct hemolytic test was subtracted from the values obtained in the indirect test in order to obtain the nearest approximation to the true 7S PFC number. Although the exact mechanism of inhibition of 19S PFC by our rabbit–anti-mouse 7S antiserum is unknown, absorption of the antiserum with purified mouse 19S antibodies reduces the 19S-inhibiting capacity of the antiserum to approximately 5% leaving the capacity intact to develop 7S PFC (21).

Inhibition Experiments.—Each mouse was immunized intravenously with a standard dose of 4×10^8 SRBC and in several experiments received simultaneously 4×10^8 ChRBC by the same route. At different time intervals thereafter half of the animals received one or several injections of anti-SRBC or anti-ChRBC antisera. The other half of the group received the same dose of normal mouse serum. At different periods after antiserum administration, the animals were bled, killed, and their number of 19S and 7S PFC against SRBC and ChRBC in the spleens were determined.

RESULTS

Indirect Hemolysis at the Cellular and Serum Level.—The inoculation of 4×10^8 sheep red blood cells intravenously cause direct and indirect hemolytic plaque-forming cells (PFC) to appear as shown in Fig. 1. A similar figure was obtained when chicken red blood cells were inoculated. The number of direct hemolytic plaque-forming cells in the spleen reached the peak around day 4 to 5 after antigen administration followed by a rapid decrease. The 19S serum titers were found to run parallel to the number of direct plaque-forming cells

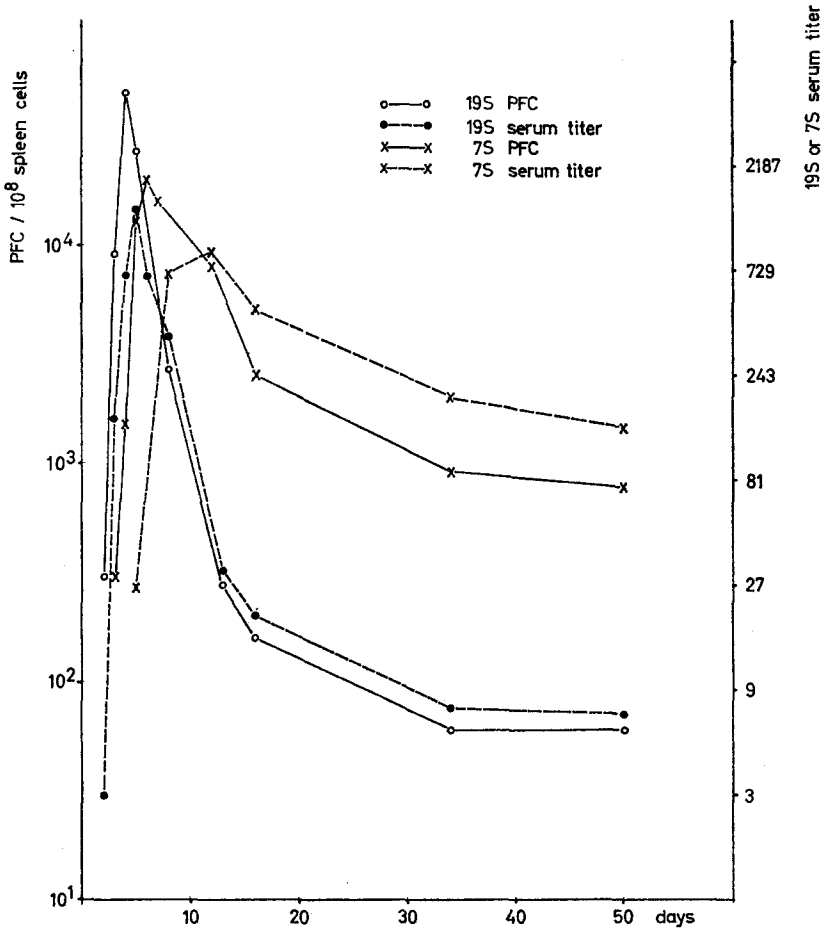

FIG. 1. Development of plaque-forming spleen cells and serum antibodies in (CBA × A.SW)F_1 hybrids after intravenous injection of 4 × 10^8 sheep red cells. 19S PFC, direct hemolytic plaque-forming cell; 7S PFC, indirect hemolytic plaque-forming cell; 19S serum titer, direct hemolytic 19S titer; and 7S titer, indirect hemolytic 7S titer. 19S and 7S antibodies were separated on Sephadex G-200 before testing. Each point represents the mean value of five mice.

in accordance with previous findings (19). The peak of indirect PFC was found around day 5 to 8 and the number was subsequently decreasing at a much slower rate than the corresponding direct PFC. Substantial numbers of indirect PFC could be found several months after a single antigen dose of this magnitude. The peak serum titer of 7S antibodies whether measured in hemagglutination or indirect or direct hemolysis was appearing around day 10 to 16 after immunization. The decay of 7S serum titers after peak titer were found to go

parallel to the decrease in the number of indirect PFC in the spleen. The discrepancy between peak time of indirect PFC and 7S serum titer is partly explained by the tendency of 7S antibodies to accumulate (half-life of passively administered hemolytic anti-SRBC 19S antibody = 8 to 12 hr; half-life of 7S antibody = 3 to 4 days).

If 19S and 7S antibodies from a mouse–anti-SRBC antiserum are separated by gel filtration and subsequently tested in direct and indirect hemolysis the indirect hemolysis shows enhancement of the direct 7S hemolytic titer by a factor of 300 as shown in Fig. 2. Instead inhibition of 19S hemolysis of at least one dilution step was a common finding. This specific potentiating effect of the 7S hemolysis in indirect hemolysis using a rabbit–anti-mouse 7S antiserum is in accordance with previous findings (14, 15).

For simplicity, the direct hemolytic plaque-forming cell will hereafter be referred to as 19S PFC and the indirect plaque-forming cell as 7S PFC (for calculation of 7S PFC in the presence of 19S PFC see Material and Methods).

Inhibition of 7S PFC by Passively Administered Antibody.—All mice received the standard dose of SRBC intravenously. In most experiments the animals received a simultaneous injection of ChRBC. At different time intervals thereafter, half of the group received passively transferred anti–SRBC or, in two experiments, anti–ChRBC antibodies, whereas the other half received the same amount of normal mouse serum. The passively administered antibodies, ranging in titers of 2-ME–resistant agglutinins from 3^8 to 3^{12}, were transferred in volumes from 0.001 to 0.5 ml in order to administer the desired number of antibody units. In certain experiments the animals received repeated inoculations of antibodies in order to prolong the action of transferred antibody. The effect of passively administered anti–SRBC antibodies on the number of 19S and 7S PFC against SRBC in the spleen using two different mouse strains is shown in Figs. 3 and 4. The results clearly indicate that both types of PFC are inhibited by the transferred antibodies even when the antiserum is administered as late as 23 days after immunization, long after the peak of both 19S and 7S synthesis has passed (see Fig. 1).

Table I summarizes the results obtained under varying experimental conditions. They show that inhibition of 7S PFC can be obtained even when passive administration of antibodies is not started until 40 days after immunization. This inhibition is specific as shown by two sets of controls. All inhibiting anti–SRBC antisera were tested at least once for their possible unspecific inhibitory effect against an unrelated antigen, ChRBC. As seen in Table I (Experiments 1, 2, and 4 to 12) no indication of depressive effect of administered anti–SRBC antibody on the number of PFC against ChRBC was seen. Furthermore, Experiments 3 and 6 in Table I where anti–ChRBC antisera were administered exclude that the anti–SRBC system might be particularly sensitive to the administration of any kind of "immune" serum. In these experiments no reduction

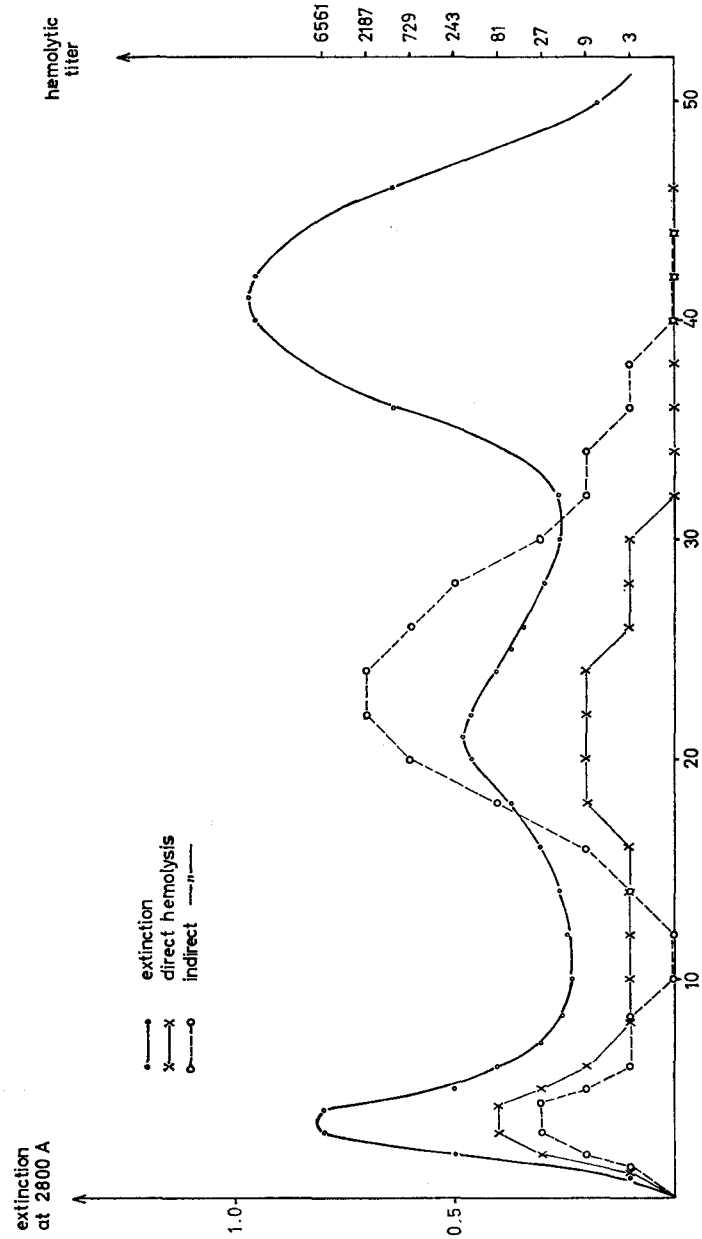

FIG. 2. Distribution of direct and indirect hemolytic antibodies in a CBA–anti-sheep red cell antiserum on day 8 after intravenous injection of 4×10^8 sheep red cells. Fractionation carried out through gel filtration on Sephadex G-200.

in the number of PFC against SRBC was seen whereas there was a drastic reduction in the number of PFC against ChRBC. The use of the same spleen cell suspension to test reactivity against two unrelated antigens excludes any nonspecific depression of antibody production by the transferred antibodies. The

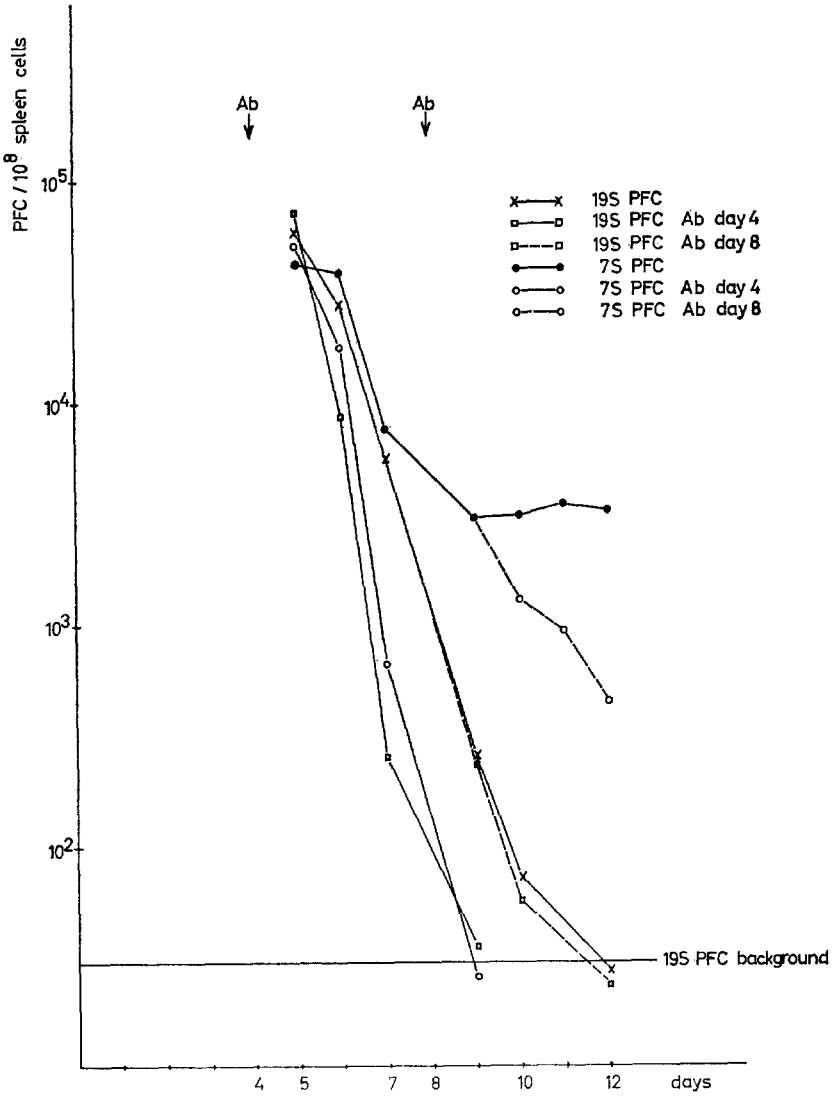

FIG. 3. Effect of passively administered anti-sheep red cell antibodies (Ab) in CBA mice on the number of 19S and 7S PFC in the spleens. All animals received an intravenous injection of sheep erythrocytes at day 0. Antiserum was administered at day 4 or day 8 after immunization. Each point represents the mean value of four mice.

7S PFC technique may however be susceptical to a technical artifact; it is conceivable that administered antibodies sticking to the cell suspension competing for the antibodies of the developing serum or complement may simulate a true decrease in number of 7S PFC. Reconstruction experiments to test this possi-

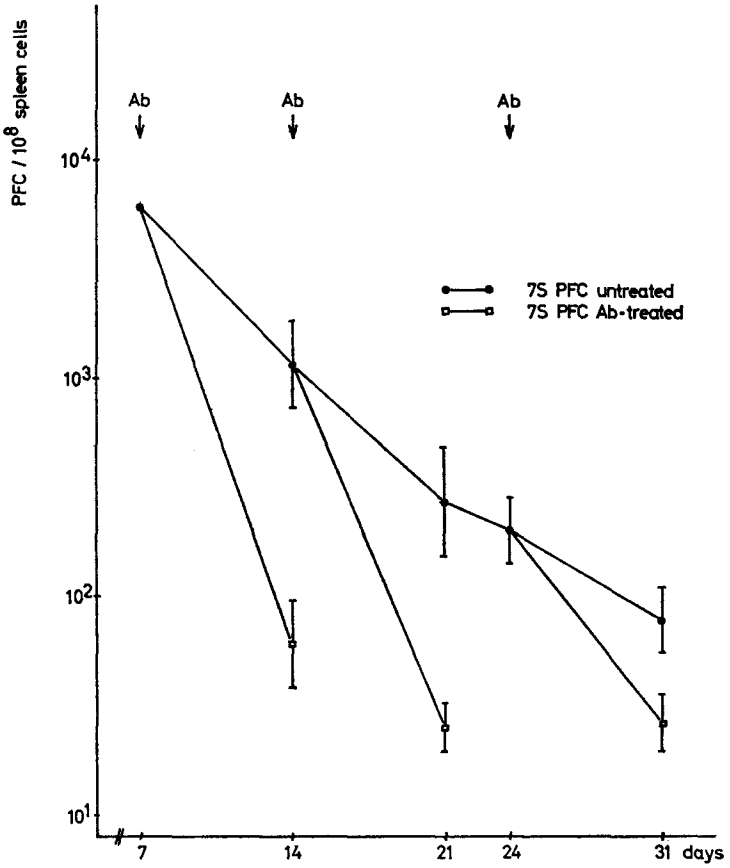

FIG. 4. Effect of passively administered anti-sheep red cell antibodies (Ab) in A.BY mice on the number of 7S plaque-forming cells in their spleens. An intravenous injection of sheep red cells was given at day 0 and antiserum was administered at day 7, 14, or 24 after immunization. Each point represents the mean value of four mice.

bility were carried out using antiserum-treated and normal spleen cell suspensions, (Table I, cells from Experiments 1, 4, and 9). Equal parts of the inhibited and normal cell suspension were tested separately or mixed together for the presence of 7S PFC and the outcome of these experiments (Table II) show that no nonspecific inhibition in vitro of 7S PFC was found.

There was a general tendency of 7S PFC to become more resistant to inhibi-

TABLE I

Inhibition of 19S and 7S PFC by Passively Administered Antibodies

Exp. No.	Strain	No. of mice*	Antiserum‡		19S PFC anti-SRBC§			7S PFC anti-SRBC§			7S PFC anti-ChRBC§		
			Time	Ratio	Controls	Antiserum-treated	Significance (Student's t test)	Controls	Antiserum-treated	Significance (Student's t test)	Controls	Antiserum-treated	Significance (Student's t test)
1	CBA/Kl	4	4:1S	50	4.757 (0.075)	4.887 (0.052)	n.s.	4.613 (0.112)	4.786 (0.056)	n.s.			
	"	4	4:2S		4.419 (0.074)	3.937 (0.147)	0.05 > P > 0.02	4.578 (0.053)	4.241 (0.184)	n.s.			
	"	4	4:3S		3.607 (0.057)	2.414 (0.244)	0.005 > P > 0.001	3.890 (0.129)	2.825 (0.121)	P > 0.001			
	"	4	4:4S		2.438 (0.363)	1.826 (0.011)	n.s.	3.126 (0.174)	2.021 (0.188)	0.01 > P > 0.005			
	"	4	4:5S		2.465 (0.027)	1.525 (0.089)	0.05 > P > 0.02	3.483 (0.229)	1.401 (0.200)	P > 0.001			
	"	4	8:2S	20	—	—	—	3.491 (0.037)	3.099 (0.055)	0.01 > P > 0.005			
	"	4	8:4S		—	—	—	3.441 (0.123)	2.640 (0.039)	0.01 > P > 0.005			
2	A.SN/Kl	3	5:3S	30	3.433 (0.132)	2.333 (0.202)	0.02 > P > 0.01	3.934 (0.048)	2.073 (0.240)	0.005 > P > 0.001	—	—	—
	"	4	5:28S		—	—	—	3.155 (0.118)	2.123 (0.220)	0.01 > P > 0.005	2.348 (0.155)	2.875 (0.134)	0.1 > P > 0.05
	"	3	10:3S	15	2.446 (0.039)	1.820 (0.042)	P > 0.001	3.382 (0.146)	2.763 (0.201)	0.1 > P > 0.05	—	—	—
	"	4	10:23S		—	—	—	3.155 (0.118)	2.199 (0.354)	0.05 > P > 0.025	2.348 (0.155)	2.443 (0.402)	n.s.
	"	3	15:4S	55	—	—	—	2.734 (0.153)	2.365 (0.082)	0.1 > P > 0.05	—	—	—
	"	4	15:17S		—	—	—	3.155 (0.118)	2.239 (0.063)	P > 0.001	2.348 (0.155)	2.387 (0.130)	n.s.
3	CBA × C57BLF$_1$	5	7:4C	30	2.186 (0.284)	1.802 (0.366)	n.s.	2.722 (0.315)	2.744 (0.178)	n.s.	2.735 (0.230)	1.644 (0.242)	0.01 > P > 0.005

4	A.BY/Kl	4	7:7S	20	—	—	—	3.067 (0.225)	1.771 (0.201)	0.005 > P > 0.001	2.386 (0.276)	2.793 (0.170)	n.s.
	"	4	14:7S	36	—	—	—	2.438 (0.254)	1.396 (0.114)	0.01 > P > 0.005	1.758 (0.214)	1.673 (0.126)	n.s.
	"	4	24:7S	40	—	—	—	2.013 (0.202)	1.537 (0.163)	0.2 > P > 0.1	1.593 (0.149)	1.587 (0.132)	n.s.
	"	4	28:7S	50	—	—	—	2.186 (0.098)	1.878 (0.119)	0.1 > P > 0.05	—	—	
5	A.BY/Kl	4	7:4S	30	2.109 (0.136)	1.426 (0.186)	0.05 > P > 0.025	3.293 (0.102)	1.396 (0.148)				
	"	4	11:4S	30	—	—	—	2.672 (0.151)	1.441 (0.201)				
	"	4	15:4S	30	—	—	—	2.948 (0.054)	2.654 (0.095)				
6	CBA × A.SWF$_1$	8	10:5S	50				3.524 (0.115)	2.432 (0.144)	P > 0.001	2.937 (0.187)	2.947 (0.223)	n.s.
	"	8	10:5C	50				3.524 (0.115)	3.519 (0.131)	n.s.	2.937 (0.187)	1.367 (0.149)	P > 0.001
7	CBA × C57BLF$_1$	10	10:5S	15	2.809 (0.149)	1.788 (0.230)	0.005 > P > 0.001	3.758 (0.185)	1.831 (0.250)	P > 0.001	1.406 (0.184)	1.795 (0.337)	n.s.
	"	10	10:35S		2.177 (0.144)	1.512 (0.180)	0.1 > P > 0.05	2.709 (0.184)	1.226 (0.144)	P > 0.001			
8	CBA × A.SWF$_1$	13	17:5S	15				2.729 (0.095)	1.940 (0.112)	P > 0.001	2.312 (0.202)	2.426 (0.260)	n.s.
9	A.BY/KL	5	18:2S	10	—	—	—	3.064 (0.150)	3.012 (0.099)	n.s.	2.150 (0.151)	2.190 (0.255)	n.s.
	"	4	18:4S		—	—	—	2.856 (0.128)	2.248 (0.030)	0.02 > P > 0.01	1.785 (0.294)	1.854 (0.250)	n.s.
10	A.BY/KL	5	21:8S	35	—	—	—	2.219 (0.123)	1.561 (0.123)	0.005 > P > 0.001	1.813 (0.324)	2.038 (0.193)	n.s.
	"	5	21:15S		—	—	—	2.259 (0.187)	1.156 (0.099)	P > 0.001	1.818 (0.282)	1.676 (0.177)	n.s.
	"	5	21:21S		—	—	—	1.746 (0.272)	1.381 (0.190)	n.s.	2.067 (0.312)	1.883 (0.300)	n.s.
11	CBA × C57BLF$_1$	5	40:5S	15	—	—	—	2.454 (0.421)	2.496 (0.293)	n.s.	2.334 (0.283)	2.561 (0.246)	n.s.
	"	5	40 + 46:5 S	15	—	—	—	2.373 (0.275)	2.616 (0.072)	n.s.	1.876 (0.353)	1.934 (0.133)	n.s.
	"	5	40 + 46 + 52:5S	15	—	—	—	2.766 (0.139)	2.145 (0.132)	0.01 > P > 0.005			
12	CBA × A.SWF$_1$	7	90 +100: 3 S	20	—	—	—	2.001 (0.150)	1.756 (0.149)	n.s.			

* No. of mice in each of the two groups.

‡ Antiserum was administered at different days after immunization as indicated by the first figure(s) and the mice were sacrificed at the day after antibody transfer as indicated by the last figure. Antiserum was transferred two or three times in Experiments 10 and 11. The antiserum administered was anti-SRBC in all experiments labeled S, anti-ChRBC when labeled C. Ratio indicates the passive increase of 2 − ME-resistant agglutinins in antiserum-treated animals compared to control animals and measured at the day of first serum transfer.

§ No. of PFC in the spleen expressed in logarithms to the base 10. Means and standard error of the means are shown. The 19S PFC values were always determined but have been excluded from the table when providing no new information.

tion with time despite keeping the same or higher relative increase in 2-ME–resistant agglutinins through passively administered antibody (see Table I, Experiments 2, 4, and 5). Finally, this resistance turned out to be almost complete as in the group where antibody was not administered until day 90 after immunization, only a weak and not significant inhibition was found despite a twentyfold increase of 2-ME–resistant agglutinins through repeatedly transferred antibodies for 13 days (Table I, Experiment 12).

TABLE II

Effect of Mixing Spleen Cell Suspensions from Normal and Antiserum-Treated Animals

Exp. No.*	Test day‡	7S PFC§			
		Control	Antiserum-treated	Mixtures, observed	Mixtures, expected
1	5:4	1500	248	848	874
	5:4	640	240	888	840
4	21:7	90	80	100	85
	21:7	40	10	38	25
	21:7	410	60	245	235
	21:7	220	30	140	125
9	18:4	770	250	560	510
	18:4	360	250	420+	345+
				3239	3039

* Experiment No. in Table I.

‡ The first figure denotes the day after immunization at which antibody was administered, the second figure refers to number of days after antibody transfer before testing.

§ Each experiments consist of one control and one antiserum-treated animal. 1:10 of a spleen was plated separately on two plates, and on two plates a mixture of 1:20 from each spleen was plated. The mean values observed and expected are shown. All PFC values have been multiplied by a factor of 10.

A few experiments were carried out in order to study in detail the time needed for passively administered antibody to start inhibiting 7S PFC. As shown in Fig. 5 no inhibition is apparent until at least 48 to 72 hr after transfer of antibodies. The number of 7S PFC in antiserum-treated and normal animals were identical at 24 hr after antibody administration, close to identical at 48 hr, but significantly different at 72 hr. Similar results were obtained in Experiments 1 and 9 in Table I, whereas longer time was needed in Experiment 11, when transfer of antibody was delayed until 40 days after immunization. In no experiment was inhibition observed before 48 hr after antibody administration irrespective of the magnitude of passive increase of 2-ME–resistant agglutinins in the sera of the recipient animals.

Inhibition of 7S Antibody Synthesis of Intact Mouse by Transferred Anti-

bodies.—In order to exclude that the antibody-induced inhibition of 7S antibody synthesis was a unique characteristic of the spleen cell population the serum titers of 2-ME–resistant agglutinins were followed in mice receiving antigen and passively administered antibody in the way previously described.

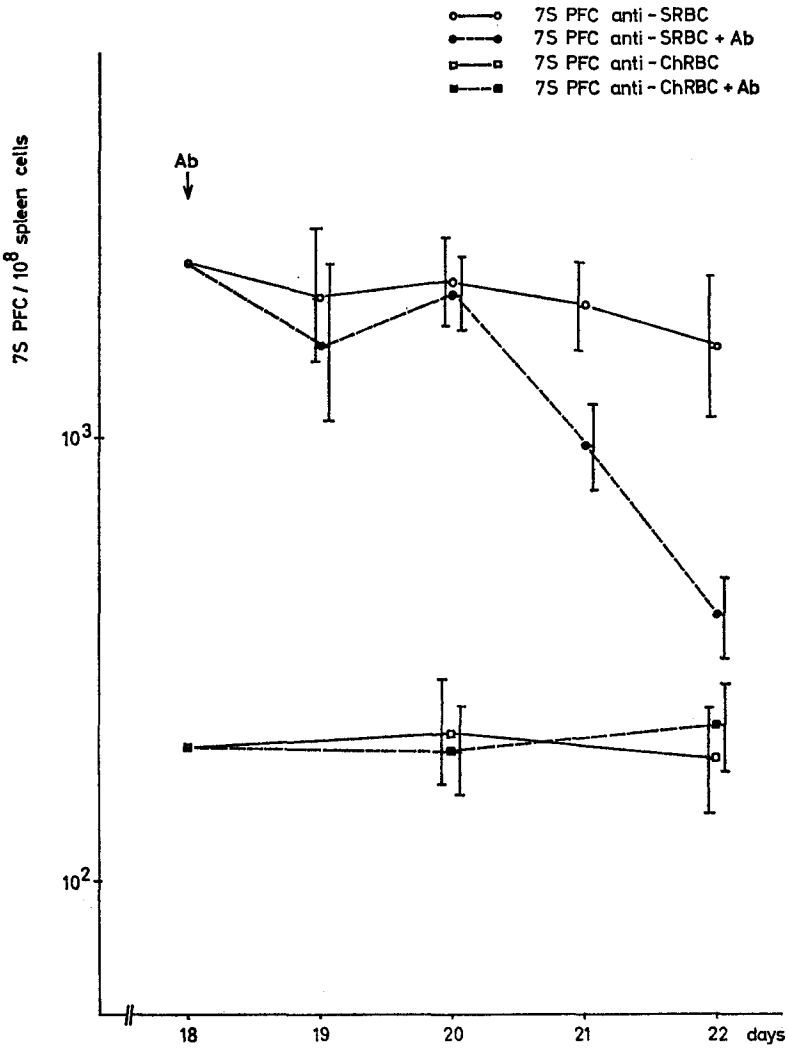

FIG. 5. Effect of passively administered anti-sheep red cell antibodies on the number of plaque-forming cells against sheep and chicken red cells in the spleens of (CBA × C57BL)F_1 hybrids. The antiserum was administered at day 18 after injection of sheep and chicken erythrocytes. Tests for plaque-forming cells were carried out every 24 hr after antiserum transfer. Each point represents the mean value of five mice.

Sufficient time was allowed for the transferred antibody to decay before serum titers were determined. Fig. 6 and Table III summarize the results obtained indicating that the passively administered antibody does inhibit 7S antibody synthesis of the intact animals as it does with 7S antibody production of the spleen. A similar increase in resistance with time to antibody-induced inhibition was also found in the 7S whole-body synthesis. Fig. 7 shows the results obtained (Table III, experiment 3), when administering antibodies at different time intervals after immunization keeping the relative, passive increase in

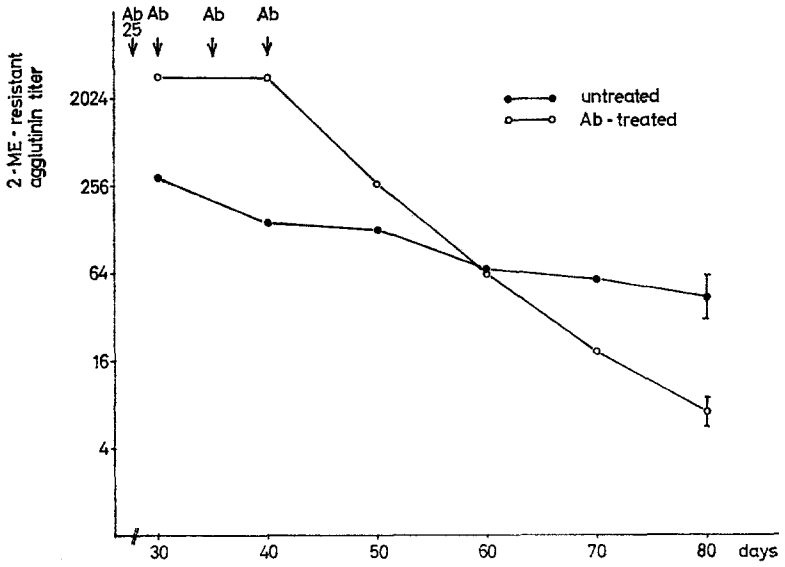

FIG. 6. Production of 2-ME-resistant 7S anti-sheep agglutinins in A.BY mice injected with sheep red cells alone or with red cells followed by antiserum at day 25, 30, 35, and 40 after immunization. Each point represents the mean of 10 mice.

2-ME-resistant agglutinins constant. 35 days after last antiserum transfer all animals were bled and tested for 2-ME-resistant agglutinins. Using a twenty-fold passive increase in 2-ME-resistant agglutinins the 7S antibody system started to become resistant to suppression by the antiserum used if this was administered later than 20 days after immunization. Thus, 7S antibody production of the intact animal seemed to react in the same ways as 7S synthesis of the spleen with regard to sensitivity to antibody-induced inhibition.

DISCUSSION

Evidence has been presented showing that 7S antibody synthesis is sensitive to antibody-induced suppression long after the peak of production has passed;

TABLE III

The Effect of Passively Administered Antibodies on the Synthesis of 7S 2-ME-Resistant Agglutinins

Exp. No.	Strain	No. of mice*	Antiserum‡		Anti-SRBC agglutinins§			Anti-ChRBC agglutinins§	
			Day	Ratio	Control $\log_{10}$ titer	Antiserum-treated $\log_{10}$ titer	Significance (Student's t test)	Control $\log_{10}$ titer	Antiserum-treated $\log_{10}$ titer
					SE	SE		SE	SE
1	A.BY/Kl	10	25 + 30 + 35 + 40:40	50	5.401 (0.494)	2.820 (0.326)	$P > 0.001$	—	—
2	A.BY/Kl	20	10:80	100	3.102 (0.586)	0.301 (0.202)	$P > 0.001$	—	—
	"	20	30 + 40 + 50:40	30	3.102 (0.586)	1.752 (0.201)	$0.02 > P > 0.01$	—	—
3	C3H/Kl	14	5:55	20	3.333 (0.302)	1.875 (0.580)	$0.02 > P > 0.01$	3.696 (0.174)	3.471 (0.190)
	"	14	10:50	20	3.333 (0.302)	1.400 (0.221)	$P > 0.001$	3.696 (0.174)	3.636 (0.129)
	"	14	15:45	20	3.333 (0.302)	1.900 (0.227)	$0.005 < P > 0.001$	3.696 (0.174)	3.730 (0.244)
	"	14	20:40	20	3.333 (0.302)	2.300 (0.366)	$0.05 < P > 0.025$	3.696 (0.174)	3.476 (0.216)
	"	14	25:35	20	3.333 (0.302)	3.375 (0.498)	—	3.696 (0.174)	3.736 (0.174)

* No. of mice in each of the two groups.

‡ In all experiments anti-SRBC antiserum was administered at the day(s) indicated before the colon and the serum titers were determined at various days thereafter as indicated by the last figure.

§ The serum titers of 2-ME-resistant agglutinins. P values calculated using Student's t test. All experiments included animals receiving only transferred antibody as controls at the day of bleeding so that no detectable passively administered antibodies were remaining.

in one experiment it was significantly inhibited by antibody administered as late as 40 days after immunization. The suppression is specific; 7S antibody synthesis is inhibited only by antibodies directed against the specific antigen while in the same animal 7S antibody synthesis against unrelated antigens is left unaffected. It has previously been found that passively administered antibody does inhibit the *induction* of both 19S and 7S antibody synthesis in the present system (8) as well as in a variety of other systems (1–7, and earlier works quoted in reference 1). When trying to inhibit 19S antibody *synthesis* it

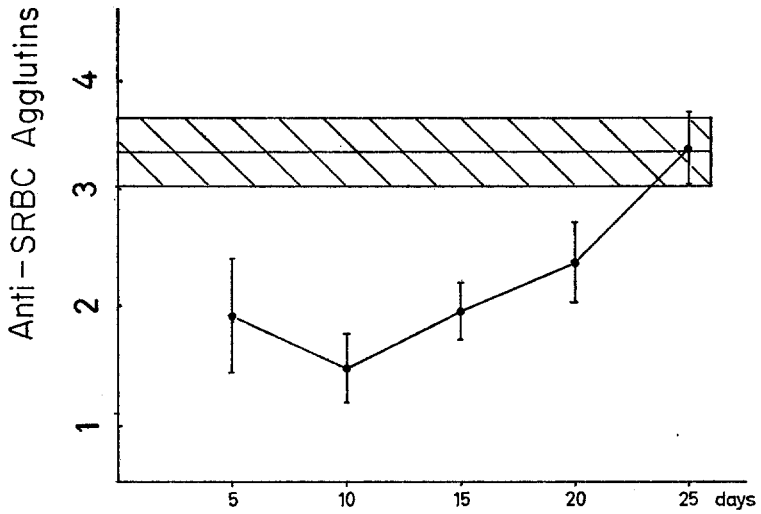

FIG. 7. Production of 2-ME–resistant 7S anti–sheep agglutinins in C3H mice injected with sheep red cells alone or with red cells followed by antiserum at day 5, 10, 15, 20, or 25 after immunization. All animals were bled and tested at day 60 after immunization. The shadowed area is indicating the mean (standard error) of the group receiving erythrocytes only. Each point represents the mean (SE) of fourteen mice.

became evident that the transferred antibodies are probably not inhibiting already committed cells, as there was a lag of 40 to 50 hr before any detectable decrease in the number of 19S PFC (8). The present data confirm this finding and extend it to the 7S system where no suppression of synthesis became apparent until at least 48 to 72 hr after antibody transfer.

It is not known in the present system whether recruitment of 7S-producing cells or division or mere survival of already synthesizing cells are playing the major roles in maintaining 7S antibody production in the postpeak period. Although the data do not allow any discrimination between these alternatives they show that at least during the 1st month(s) after induction 7S antibody synthesis is still dependent upon something which in a specific way is removed from the system by passively transferred antibody. The exact mechanism

through which antibodies suppress the immune response is unknown but there is no evidence to exclude that they act by combining with antigenic determinants. It has been reported that flagellar antigens of bacteria are not detectable in the specific antibody-producing cells (22). If erythrocyte antigens are not present in the synthesizing cells it would follow that antigen localized in some other cell type(s) would be responsible for stimulating 7S antibody synthesis according to the above mentioned alternatives. The fact that inhibition of 7S antibody production did not become evident until 48 to 72 hr after antibody transfer would indicate a corresponding lifetime of these specific stimulating factors.

It has previously been found that 19S antibody synthesis is inhibited more easily by 7S antibodies than by 19S antibodies (6, 8). In the present system different batches of 7S antibodies with approximately the same titers of 2-ME–resistant agglutinins have been found to differ markedly with regard to capacity to inhibit 7S antibody synthesis, being increasingly efficient with increasing number of immunizations (references 8 and 21, and present article). Similar findings have been reported by Finkelstein and Uhr (6) when testing 7S antisera with the same phage-neutralizing activity in vitro for their capacity to inhibit the immune response in vivo. These results indicate a change with time within or between type of 7S antibodies being produced after immunization. This is consistent with the known existence of a number of 7S immunoglobulin classes (23) and the changes of characteristics within an immunoglobulin class taking place with time after immunization (24). One might speculate that a gradual change into production of more efficient inhibiting antibodies not necessarily correlated to changes in the titer of 2-ME–resistant agglutinins could explain the increasing resistance with time to antibody-induced suppression in the present 7S system. Thus, antibody transfer causing a passive increase in 2-ME–resistant agglutinins might not be correlated to a corresponding increase in "efficient inhibiting" antibodies thus failing to inhibit 7S antibody synthesis. However, an alternative explanation of the increasing resistance of the 7S antibody system to antibody-induced inhibition would be the accumulation of long-lived 7S-producing cells like certain plasma cells in the rat (25), which may survive for prolonged periods in the absence of antigen. Studies using radioautography are in progress trying to solve this question.

Taken together the present data suggest that antigen is of major importance in maintaining and stabilizing 7S antibody synthesis during a prolonged period after induction of 7S antibody production against erythrocyte antigens. They also emphasize the role of antibody functioning as a feedback factor during immune response both with regard to 19S and 7S antibody synthesis.

SUMMARY

The specific suppressing activity of passively administered antibody on 7S antibody synthesis against sheep and chicken red blood cells has been investi-

gated at the cellular level using the indirect hemolytic agar-plaque technique. 7S antibody production was found to be sensitive to antibody-induced suppression. No inhibitory effect of transferred antibody was seen until 48 to 72 hr after administration. This indicates that the action of antibody is not by direct suppression of synthesis of already committed cells but rather by removal from the system of the stimulus for maintenance of 7S synthesis. The sensitivity of the 7S system to inhibition decreases with time after immunization but significant specific suppression could still be obtained if transfer of antibody was delayed until 40 days after immunization. The present findings emphasize the role of antibody as a feedback factor during a substantial post-peak period of 7S antibody synthesis and suggest an important role of antigen in stabilizing the 7S antibody production.

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