REGULATION OF CELLULAR ANTIBODY SYNTHESIS

Cellular 7S Production and Longevity of 7S Antigen-Sensitive Cells in the Absence of Antibody Feedback

By GÖRAN MÖLLER, M.D.

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

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Antibody synthesis is efficiently regulated with regard to the maximum number of antibody-producing cells reached after immunization, in spite of considerable variation in the dose of antigen used and the number of injections given. The existence of homeostatic mechanisms is also suggested by the sequential appearance of different antibody classes, by the predictable increase of antibody avidity, and by the increase of the number of antibody specificities with time after immunization with complex antigens. In some immunological systems the main factor which appears to restrict antibody synthesis is related to the rapid decay of the antigen; in particular this seems to be true for protein antigens (1). However, other regulating mechanisms must be postulated with antigens that persist for long time periods. Lipopolysaccharides and sheep red blood cells (SRBC) belong to this category and have been shown to remain immunogenic in their primary host for several months and weeks, respectively (2). It seems likely that the ability of antibodies to suppress their own synthesis constitutes the main homeostatic mechanism in these cases (for review see reference 3). Not only passively transfused hyperimmune sera, but also endogenously produced antibodies in the primary response are competent to suppress antibody synthesis (3).[1] Furthermore, passive transfer of antibodies a long time after induction of immunity may suppress both 19S and 7S synthesis (4–9). Cellular 19S antibody synthesis against a lipopolysaccharide antigen may even cycle repeatedly.[2] This is most likely caused by a regular feedback suppression of synthesis by antibody, followed by renewed antigen stimulation when the suppressing antibodies are catabolized (10). Taken together, the various findings suggest that antibody suppression represents a major feedback mechanism controlling the number of antibody-pro-

[1] Morris, A., and G. Möller. Regulation of cellular antibody synthesis. Effect of adoptively transferred immune spleen cells on cellular antibody production. Manuscript to be published.

[2] Britton, S., and G. Möller. Regulation of antibody synthesis against *E. coli* endotoxin. I. Suppressive effect of endogenously produced and passively transferred antibodies. Manuscript to be published.

ducing cells against antigens, which persist in an immunogenic form for long time periods.

According to this concept, it would be expected that stimulation of antibody production in an environment lacking suppressive antibodies would lead to excessive antibody synthesis and to uninhibited multiplication of committed cells. In the present experiments this was tested by inoculating preimmunized spleen cells mixed with the antigen (sheep red blood cells) into sublethally irradiated, nonimmune syngeneic recipients. As will be shown this resulted in spleen cell populations which to a large extent were made up of specifically antibody-producing cells, which made it possible to study the proliferative capacity of such populations subsequent to repeated stimulation with antigen over long time periods; e.g., to investigate longevity of immunological memory.

Materials and Methods

Mice of the inbred strains CBA, C57BL/10.5M (5M), A, A.BY., C57BL, C57L, and F_1 hybrids between these strains were used. The animals within each experiment and in all transfers were of the same sex. They were bred and kept as described previously (11). Whole-body X-irradiation was carried out by giving the animals 600 or 900 R at a dose rate of 62 R/min. The X-rays were generated in a Siemens X-ray machine at 185 kv and 15 ma and were filtered by 0.5 mm Cu.

The agar plaque technique was carried out as described by Jerne and Nordin (12) for the detection of direct (19S) plaque-forming cells (PFC). Indirect PFC were detected by the method of Dresser and Wortis (13), and of Sterzl and Riha (14). A rabbit anti-mouse gamma globulin serum was produced by immunizing rabbits with *Salmonella adelaide* bacteria coated with mouse antibodies against their H antigen. The bacteria were washed after treatment with the mouse antiserum and injected intravenously. At least three weekly injections were given prior to bleeding, but in most experiments the sera were taken after 10 injections or more. 3 ml of this serum diluted 1/100 were added to each plate, which had been incubated before at 37°C for 1 hr. After 30 min at 37°C the serum was replaced with 3 ml guinea pig complement (Sclavo, Italy), diluted 1/10, and incubated for another 30 min at 37°C. The plates were left in a refrigerator overnight and were subsequently stained as described by Jerne and Nordin (12).

Cell suspensions were prepared from spleens by pressing them through a 60 mesh stainless steel screen into Eagle's medium in Earle's solution. The suspensions were washed once and thereafter made up to the desired volume in the same medium.

Serology.—Hemolysis in the presence of guinea pig complement diluted 1/20 and hemagglutination in the presence of polyvinylpyrrolidone (PVP) was carried out as before (11).

Experimental procedure.—Immune spleen cells were diluted to 50×10^6 or 100×10^6 cells per ml and 0.25 ml packed SRBC were added to each ml of cell suspension. As a rule 0.25 ml of this suspension was inoculated intravenously into X-irradiated recipients. The recipients were usually killed after 7 days, and the number of 7S and/or 19S plaque-forming spleen cells was determined.

RESULTS

Cellular 7S Production in X-irradiated, Nonimmune Recipients.—To test the hypothesis that antibodies against SRBC function in a feedback system to restrict the increase of the number of antibody-producing cells, immune spleen

cells were mixed with SRBC and inoculated into X-irradiated (600 R), syngeneic and nonimmunized recipients. The X-ray dose was sufficient to suppress induction of antibody synthesis in the host as will be shown below.

The normal proportion of 7S PFC against SRBC in the spleens of hyperimmunized animals varies between 0.01–0.5%, as a rule. 7 days after the inoculation of 10^7 immune spleen cells mixed with SRBC into irradiated recipients the usual mean proportion of 7S PFC varied between 1 and 56% (Table I), and the total number of 7S PFC per spleen in the irradiated recipients was higher than 10^6 in most cases and reached up to 10×10^6 PFC in some experiments. When the inoculum dose was increased to $40–50 \times 10^6$ immune cells the mean proportion of 7S PFC often increased even more and constituted between 1 and 70% of the spleen population and the total number 7S PFC/spleen varied between 0.3 and 60×10^6. In some animals all spleen cells were found to be antibody producing (Table I).

In several experiments the number of 19S PFC was also determined and was usually found to be higher than in the hyperimmune donors, but nevertheless they only constituted a small fraction (less than 1%) of the total number of PFC (Table I). It was not considered necessary, therefore, to correct the number of 7S PFC for 19S PFC as is usually done.

Controls showed that the PFC were derived from the inoculated cells and not from host cells, since injection of SRBC into irradiated animals did not lead to detectable production of 19S or 7S PFC (for typical results see Table III). Furthermore, the increase of the number of 7S PFC was entirely dependent upon stimulation of the spleen cells with SRBC, since the number of PFC in recipients of immune spleen cells, which had not been stimulated with SRBC were the same or only slightly above the normal background in untreated nonirradiated animals (Table I).

The number of 7S PFC increased with time after inoculation (Table I). 4 days after inoculation it varied between 4.6 and 14.5×10^3 (0.03 and 0.33%), but increased to higher values after 7 days. The proportion of 7S PFC found after 4 days was within the values found in nonirradiated hyperimmune recipients. As shown in Fig. 1, the number of 7S PFC/spleen increased in parallel from day 4 to day 7 in three different experiments performed in different mouse strains. The mean doubling time was 9.6 hr and the mean curve intercepted at day 0 with the value of one for the number of 7S PFC. As will be discussed below, this value is unlikely, since 10^7 immune cells were inoculated and even if only a small minority of the antigen-sensitive cells settled down in the spleen, their number most likely exceeds one. Consequently, the doubling time must be longer between day 0–4 than between days 4–7, or there is a lag phase before the development of the 7S PFC.

The serum antibody titers in the irradiated recipients were also determined and found to be considerably higher than in hyperimmune animals. As seen in Table II the $\log_2$ titers in agglutination after 2-ME treatment and in hemolysin were higher than 15 and sometimes reached more than 25.

It seemed possible that the large number of 7S PFC found in the transfer system did not reflect active synthesis of antibodies, but was caused by passive adsorption of antibodies onto nonproducing cells or cellular debris formed after irradiation. Although the marked increase serum antibody production argues for active synthesis, it was

TABLE I

19S and 7S PFC in Nonimmune and Irradiated Mice Given Antigen-Stimulated Spleen Cells from Donors Hyperimmunized against SRBC

Exp No.	Strain	X-Ray dose	No. of cells transferred	Immunized with SRBC	PFC determined at day	Mean No. of 19S PFC/spleen*	% of 19S PFC‡	Mean No. of 7S PFC/spleen*	% of 7S PFC‡
		R							
1	A × C57Bl	600	10×10^6	−	4	<10 (all <10)		<10 (all <10)	
	"	600	10×10^6	−	7	244 (90–475)	0.001 (0.0005–0.0020)	934 (885–1200)	0.005 (0.003–0.006)
	"	600	10×10^6	+	4	1171 (870–2,250)	0.06 (0.04–0.09)	4608 (1040–7080)	0.29 (0.13–0.65)
	"	600	10×10^6	+	7	68,183 (46,530–93,000)	0.44 (0.37–0.58)	1.12×10^6 (1.05–1.17)	5.94 (4.69–6.65)
2	A × C57L	600	10×10^6	−	4	<20 (all <20)		<20 (all <20)	
	"	600	10×10^6	−	7	<20 (all <20)		<20 (all <20)	
	"	600	$10 \times \frac{1}{2} 10^6$	+	4	160 (70–350)	0.005 (0.001–0.013)	14,560 (7880–20,040)	0.33 (0.23–0.49)
	"	600	$10 \times \frac{1}{2} 10^6$	+	7	5340 (3140–7760)	0.019 (0.011–0.028)	1.23×10^6 (0.69–1.72)	4.60 (2.45–7.05)
3	A × C57Bl	600	40×10^6	−	7	n.t.	n.t.	593 (60–1125)	0.03 (0.0007–0.06)
	"	600	40×10^6	+	7	n.t.	n.t.	7.76×10^6 (3.6–10.98)	12.75 (4.17–17.60)
4	CBA	600	10×10^6	+	4	373 (285–500)	0.002 (0.001–0.002)	6632 (5630–7615)	0.03 (0.02–0.04)
	"	600	10×10^6	+	7	<2000 (all <2000)	<0.004	1.73×10^6 (1.42–1.94)	3.7 (3.0–4.4)
5	CBA	700	50×10^6	+	7	0.347×10^6 (0.193–0.473)	0.62 (0.46–0.78)	38.82×10^6 (24.09–60.36)	69.94 (52.63–99.28)
6	CBA	600	10×10^6	+	7	11,200 (4800–22,100)	0.48 (0.22–0.84)	0.9×10^6 (0.7–1.0)	40.8 (28.6–53.8)
7	A	600	10×10^6	+	7	10,350 (9150–11,150)	0.93 (0.72–1.14)	0.55×10^6 (0.5–0.6)	50.0 (37.5–62.5)
8	CBA	600	10×10^6	+	7	198 (50–395)	0.002 (0.0003–0.005)	$>6.4 \times 10^6$ (6.1–>7.0)	>56.4 (34.6–87.5)
9	CBA	600	10×10^6	+	7	12,990 (1700–26,150)	0.066 (0.012–0.11)	0.94×10^6 (0.7–1.0)	9.47 (2.84–31.3)
10	A.BY	600	60×10^6	+	7	23,390 (7320–50,000)	0.08 (0.7–0.10)	1.74×10^6 (0.32–2.80)	7.34 (3.29–15.49)
11	CBA	600	10×10^6	+	7	n.t.	n.t.	5.50×10^6 (4.42–9.32)	9.13 (5.06–14.56)
12	CBA	600	10×10^6	+	7	n.t.	n.t.	4.70×10^6 (4.30–5.10)	5.54 (5.38–5.67)

TABLE I—*concluded*

Exp No.	Strain	X-Ray dose	No. of cells transferred	Immunized with SRBC	PFC determined at day	Mean No. of 19S PFC/spleen*	% of 19S PFC‡	Mean No. of 7S PFC/spleen*	% of 7S PFC‡
		R							
13	CBA	600	10×10^6	+	7	n.t.	n.t.	3.52×10^6 (1.68–5.76)	4.55 (2.97–7.20)
15	CBA	600	40×10^6	+	7	2480 (200–7150)	0.016 (0.001–0.040)	1.05×10^6 (0.25–1.49)	7.9 (3.5–13.4)
16	CBA	600	10×10^6	+	7	n.t.	n.t.	1.84×10^6 (0.57–3.72)	2.49 (0.94–5.81)
17	CBA	600	10×10^6	+	7	59,890 (32,200–100,000)	0.105 (0.45–0.200)	6.86×10^6 (1.78–10.0)	11.78 (2.78–17.86)
18	CBA	600	10×10^6	+	7	n.t.	n.t.	3.76×10^6 (1.13–8.31)	7.83 (1.77–17.50)
19	CBA	600	10×10^6	+	7	n.t.	n.t.	2.28×10^6 (0.49–4.20)	3.52 (0.96–5.26)
20	A × 5M	900	50×10^6	+	7	25 (0–50)	0.002 (0–0.103)	0.29×10^6 (0.19–0.39)	2.41 (2.38–2.44)
21	A × 5M	600	40×10^6	+	7	29,540 (20,175–39,600)	0.07 (0.04–0.10)	0.45×10^6 (0.28–0.530	0.99 (0.70–1.10)

n.t., not tested.
* Figures within parenthesis indicate range.
‡ % PFC, No. of PFC/spleen × 100 divided by No. of spleen cells.

tried to suppress the 7S PFC by treating the spleen cells with puromycin (200 μg/ml) in vitro for 30 min at 37°C and thereafter determine the number of 7S PFC in the presence of puromycin. After treatment with puromycin all 7S PFC disappeared, whereas the expected numbers were found in untreated controls. Thus, plaque formation was caused by active antibody synthesis.

Cellular 7S Production in Actively or Passively Immunized Irradiated Recipients.—The previous findings show that 7S antibody-producing cells from immune animals have a marked ability to increase in number to such an extent that they may constitute the major part of the spleen cell population, after they have been transferred into irradiated nonimmune recipients together with the specific antigen. Thus, the comparatively low proportion of 7S PFC found in hyperimmune animals cannot be caused by an inherent inability of 7S-producing cells to increase in number by multiplication or nonmitotic differentiation after antigen stimulation. If antibody feedback is responsible for the limitation of the number of 7S PFC in hyperimmune animals, it is to be expected that transfer of immune cells with SRBC into already immunized and X-irradiated recipients would lead to a lower number of 7S PFC 7 days

later, as compared with the number in nonimmune hosts. Experiments were performed to test this idea.

Immune cells mixed with SRBC were injected into irradiated (600 R) recipients which had been previously hyperimmunized against SRBC. As a comparison nonimmune animals were similarly treated. Controls included irradiated hyperimmune and nonimmune animals, respectively, which were given only SRBC. As shown in Table III the nonimmune controls did not develop any 7S PFC. The number of 7S

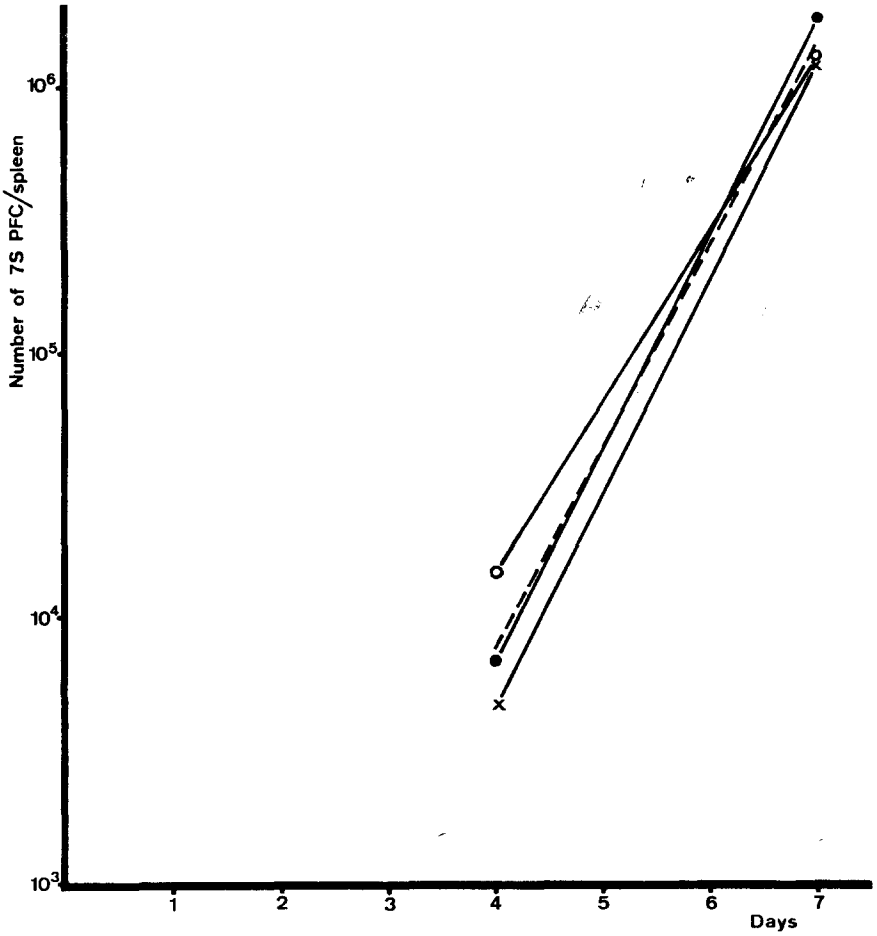

FIG. 1. Number of 7S PFC/spleen against SRBC 4 and 7 days after the intraveous inoculation of 10×10^6 hyperimmune spleen cells mixed with antigen into irradiated (600 R) nonimmune syngeneic recipients. The figure shows three separate experiments performed in (A × C57BL)F_1 (×——×), (A × C57L)F_1 (○——○), and CBA (●——●) mice. The dotted line indicates the exponential mean curve of the three experiments.

PFC in immune, irradiated, and SRBC-injected controls varied between 230–3500 PFC per spleen, but it was still much lower than in hyperimmune nonirradiated animals. Thus, irradiation efficiently suppressed active antibody synthesis. This is in accordance with previous findings (2). The proportion of 7S PFC in nonimmune

TABLE II

Hemolysin and 2-ME-Resistant Agglutinin Titers in Irradiated Nonimmune Mice Given Spleen Cells Immunized against SRBC

Exp. No.	Strain	X-Ray dose	No. of cells transferred	Immunized with SRBC	PFC and titers determined at day	Mean No. of 19S PFC/spleen	Mean No. of 7S PFC/spleen	Mean $\log_2$ titers in	
								hemolysis*	agglutination*
		R							
1	A × C57Bl	600	10×10^6	−	7	244	934	8.5 (8–10)	4.0 (3–5)
	A × C57Bl	600	10×10^6	+	7	68,183	1.12×10^6	>23.3 (21–>24)	21.8 (20–24)
2	CBA	600	10×10^6	+	4	373	6622	—	15.0 (14–16)
	CBA	600	10×10^6	+	7	<2000	16.4×10^6	—	22.7 (21–25)
3	A × 5M	600	40×10^6	+	7	29,540	0.45×10^6	19.0 (17–21)	15.5 (14–18)
4	A × C57Bl	600	40×10^6	−	7	—	593	11.5 (10–13)	12.0 (10–14)
	A × C57Bl	600	40×10^6	+	7	—	7.76×10^6	>25.0 (25–>25)	21.3 (19–25)
5	A × C57L	600	10×10^6	−	4	<20	<20	<3.0	<3.0
	A × C57L	600	10×10^6	+	4	160	13,345	7.3 (5–9)	8.8 (8–10)
	A × C57L	600	10×10^6	−	7	<20	<20	5.0 (4–8)	5.8 (5–8)
	A × C57L	600	10×10^6	+	7	5340	1.23×10^6	20.0 (18–23)	21.3 (21–22)

* Figures within parenthesis indicate range of reciprocal of $\log_2$ titers.

recipients of stimulated spleen cells was high (2.8–17.9%) as expected (Table III), whereas it was considerably lower in immunized recipients (0.07–3.0%). The degree of suppression of the proportion of 7S PFC varied between 89 and 98% and the total number of 7S PFC per spleen was even more suppressed (94–100%). 19S PFC in immune recipients were considerably lower than in nonimmune recipients, the percentage of suppression with regard to the total number being 69% and with respect to

TABLE III

Suppression of Cellular 19S and 7S Production against SRBC after Transfer of Immune Stimulated Spleen Cells into Hyperimmune and Irradiated Recipients

Exp. No.	Strain	Immune status of recipients	X-Ray dose	No. of cells transferred	Immunized with SRBC	PFC determined at day	No. of 19S PFC per spleen	% 19S PFC*	No. of 7S PFC per spleen	% of 7S PFC*	% suppression in immune recipients as compared with nonimmune hosts of			
											19S PFC/ spleen	% 19S PFC	7S PFC/ spleen	% 7S PFC
			R											
1	CBA‡	NI§	600	10×10^6	+	7	59,890 (32,200–>100,000)	0.105 (0.045–0.20)	6.86×10^6 (1.78–10.0)	11.78 (2.78–17.86)				
	"	HI‖	600	—	+	7	1528 (1200–1855)	0.10 (0.03–0.12)	880 (230–1530)	0.055 (0.01–0.10)				
	"	HI	600	10×10^6	+	7	18,465 (10,125–28,750)	0.07 (0.03–0.18)	0.395×10^6 (0.188–0.793)	1.51 (0.47–2.97)	69.2	33.3	94.2	88.7
2	CBA	NI	600	—	+	7	n.t.¶	n.t.	0					
	"	HI	600	—	+	7	n.t.	n.t.	2943 (2260–3560)	0.065 (0.028–0.094)				
	"	NI	600	10×10^6	+	7	n.t.	n.t.	4.7×10^6 (4.3–5.1)	5.54 (5.38–5.69)				
	"	HI	600	10×10^6	+	7	n.t.	n.t.	11,500 (6500–19,000)	0.12 (0.067–0.17)			99.8	97.8

* % PFC, No. of PFC per spleen × 100 divided by No. of spleen cells.

‡ Four mice per group.

§ NI, nonimmune.

‖ HI, hyperimmunized.

¶ n.t., not tested.

the proportion 19S PFC being 33%. It seems likely that the preexisting antibodies in the immunized animals were responsible for suppression of the development of 7S PFC.

It is to be expected that passive transfer of hyperimmune sera also would suppress cellular antibody synthesis in irradiated nonimmune recipients in this system. Direct experiments confirmed this (Table IV). Hyperimmune sera, derived from animals immunized repeatedly against SRBC, were injected intraperitoneally into recipients which subsequently were given immune cells mixed with SRBC. The antibodies were injected intraperitoneally 1 hr after the intravenous inoculation of the spleen cells. The antibodies were derived from animals immunized for a longer time period and more often than the donors of the transferred cells. The antiserum-treated recipients developed fewer 19S and 7S PFC, the degree of suppression in comparison with non-antibody-treated irradiated controls ranging from 53 to 70% with regard to the proportion of 7S PFC from 80 to 85% with respect to the total number. 19S antibody synthesis was suppressed to an equal extent (Table IV).

Serial Transfer of Antigen-Sensitive Cells in Irradiated Hosts.—The increase of the number of 7S PFC after transfer of immune cells mixed with antigen is most likely due to proliferation of antigen-reactive precursor cells. Although the antigen-sensitive cells may be identical with the 7S PFC present in the inoculum, various findings suggest that they are different. Thus, antigen-sensitive cells studied by an analogous transfer system can be efficiently suppressed by a heterologous antilymphocyte serum, whereas 7S PFC are unaffected or only slightly suppressed.[3] Furthermore, passive transfer of specific antibodies effectively inhibits the development of antibody-producing cells, whereas antigen-sensitive cells are relatively unaffected, as revealed by a normal secondary response (for references see reference 3). Finally, physical separation of antibody-producing cells from a lymphoid cell population by glasswool filtration does not remove the antigen-sensitive cells capable of responding to antigen in a secondary host.[4] Therefore, it seemed possible to study the proliferative capacity and the longevity of antigen-sensitive cells by serial transfers of immune cell populations in irradiated recipients.

As shown above very few 7S PFC were recovered in the spleens of animals given immune cells alone (without antigen stimulation). In order to test whether such spleens contained antigen-sensitive cells, they were transferred into irradiated recipients in the presence of SRBC. Several experiments of this type were performed, but it was constantly found that a second transfer with antigen, carried out 7 days after the first, did not lead to reestablishment of antibody production. Therefore, it would appear that antigen-sensitive cells were rapidly lost in the secondary host in the absence of antigen.

[3] Möller, G., and C. Zukoski. Suppression by antilymphocyte serum of 7S memory cells. Manuscript to be published.

[4] Wigzell, H. Personal communication.

TABLE IV

Suppression of Cellular 19S and 7S Synthesis by Passively Administered Antibody in Nonimmune and Irradiated Mice Given Antigen (SRBC) Stimulated Immune Spleen Cells*

Exp. No.	Strain	X-Ray dose	No. of cells transferred	Antibody treatment‡	PFC determined at day	Mean No. of 19S PFC/spleen§	% 19S PFC‖	Mean no. of 7S PFC/spleen§	% 7S PFC‖	% suppression in antibody-treated as compared with untreated host with regard to			
										19S PFC/ spleen	% 19S PFC	7S PFC/ spleen	% 7S PFC
		R											
1	CBA	600	10×10^6	—	7	12,300 (4500–28,500)	0.04 (0.002–0.089)	0.24×10^6 (0.19–0.29)	0.93 (0.68–1.32)				
	CBA	600	10×10^6	+	7	380 (150–750)	0.0019 (0.0006–0.0028)	0.047×10^6 (0.025–0.075)	0.28 (0.12–0.67)	98.5	95.3	80.4	69.9
2	C57L	600	10×10^6	—	7	3081 (1625–7200)	0.005 (0.002–0.008)	0.105×10^6 (0.089–0.142)	0.17 (0.14–0.20)				
	C57L	600	10×10^6	+	7	1030 (600–1500)	0.004 (0.001–0.008)	0.019×10^6 (0.017–0.021)	0.08 (0.02–0.22)	66.2	20.0	84.9	52.9

* Mouse anti-sheep red blood cells serum from hyperimmune animals.

‡ 0.2 or 0.3 ml antiserum was given intraperitoneally 1 hr after the intravenous injection of the spleen cells.

§ Figures within parenthesis indicate range.

‖ % PFC, No. of PFC/spleen × 100 divided by No. of spleen cells.

TABLE V

Development of 7S PFC after Serial Transfers in Nonimmune and Irradiated Hosts of Repeatedly Antigen-Stimulated Spleen Cells from Donors Originally Hyperimmunized against SRBC*

Exp No.	Strain	Passage 1		Passage 2		Passage 3		Passage 4		Passage 5		Passage 6		Passage 7		Passage 8	
		7S PFC/ spleen	% 7S PFC	7S PFC/ spleen	% 7S PFC	7S PFC/ spleen	% 7S PFC	7S PFC/ spleen	% 7S PFC	7S PFC/ spleen	% 7S PFC	7S PFC/ spleen	% 7S PFC	7S PFC/ spleen	% 7S PFC	7S PFC spleen	% 7S PFC
1	CBA	1.05×10^6 (0.25–1.49)	7.9 (3.5–13.4)	n.t.	n.t.	0.84×10^6 (0.5–1.02)	2.60 (2.19–3.04)	2.86×10^6 (0.78–8.0)	9.75 (3.89–20.0)	0.115×10^6 (0.06–0.22)	0.84 (0.49–1.52)	8300 (7000–10,000)	0.05 (0.04–0.06)	214 (0–456)	0.012 (0–0.029)	n.t.	n.t.
2	CBA	1.89×10^6 (0.57–3.72)	2.49 (0.94–5.81)	0.38×10^6 (0.03–0.91)	4.05 (0.25–14.2)	0.42×10^6 (0.23–0.61)	2.24 (2.12–2.35)	n.t.	n.t.	10,941 (2250–37,100)	0.081 (0.029–0.27)	<1000 (all <1000)	<0.01	n.t.	n.t.	n.t.	n.t.
3	CBA	$>6.4 \times 10^6$ (6.1–>7.0)	>56.4 (34.6–>87.5)	2.65×10^6 (2.30–4.44)	18.3 (8.0–27.4)	4.23×10^6 (4.3–7.0)	10.3 (6.4–19.2)	2.57×10^6 (0.21–5.47)	12.0 (3.6–28.5)	0.09×10^6 (0.03–0.12)	0.90 (0.38–1.37)	0.45×10^6 (0.35–0.59)	1.46 (0.8–2.4)	<1000 (all <1000)	<0.01	<1000	<0.01
4	CBA	0.94×10^6 (0.70–1.00)	9.47 (2.84–31.3)	0.06×10^6 (0.02–0.09)	0.78 (0.3–1.4)	0.41×10^6 (0.33–0.58)	1.93 (0.9–3.0)	0.013×10^6 (0.008–0.015)	0.13 (0.05–0.20)	2750 (500–7500)	0.013 (0.001–0.026)	<10 (all <10)	<0.0001	n.t.	n.t.	n.t.	n.t.
5	CBA	0.94×10^6 (0.70–1.00)	9.47 (2.84–31.3)	0.12×10^6 (0.09–0.17)	3.13 (1.96–5.31)	0.858×10^6 (0.31–1.39)	9.98 (3.8–14.2)	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.
6	CBA	$>7.0 \times 10^6$ (all >7.0)	>18.5 (>13.9–>21.9)	3.17×10^6 (0.54–5.8)	26.3 (12.2–40.3)	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.
7	CBA	38.8×10^6 (24.1–60.4)	69.9 (52.6–99.3)	0.90×10^6 (0.02–1.74)	4.47 (1.4–6.5)	1.18×10^6 (0.50–1.70)	44.3 (1.3–100.0)	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.
8	CBA	3.76×10^6 (1.13–8.31)	7.85 (1.77–17.50)	1.63×10^6 (1.35–2.13)	3.13 (2.49–3.35)	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.

* Five to seven mice were irradiated with 600 R and inoculated with $5–20 \times 10^6$ spleen cells.

To study the longevity of antigen-sensitive cells in the presence of antigen, serial transfers of antigen-stimulated immune cells were carried out. Such transfers were invariably successful for the first two to four generations and the number of 7S PFC in the recipient spleens after 7 days was within the expected range (Table V). However, during the fifth to sixth transfer the number of 7S PFC started to decline rapidly.

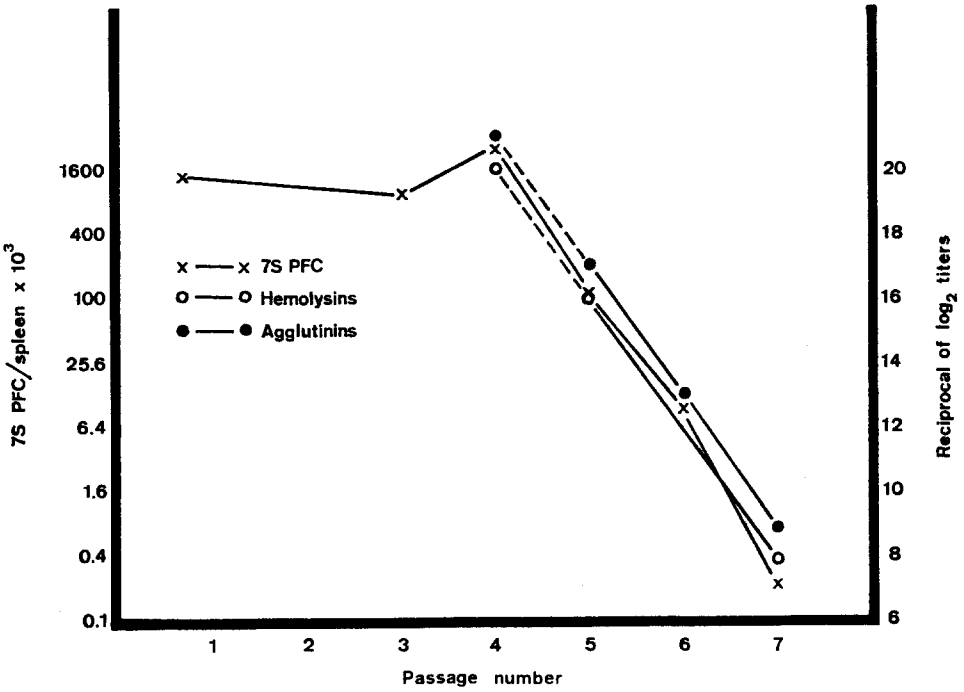

FIG. 2. 7S PFC/spleen, hemolytic, and hemagglutinating titers against SRBC during repeated passages of antigen-stimulated CBA spleen cells from hyperimmune donors in irradiated (600 R) nonimmune CBA recipients. Titers and 7S PFC were determined 7 days after transfer. Each point represents the mean value of at least five animals. The dotted lines in the curves for serum antibody titers between passage 4 and 5 indicate that the values for passage 4 were not determined in this experiment, but derived from an analogous experiment in the same strain, in which the number of 7S PFC was similar.

The decrease occurred simultaneously in all five to six recipients used in each group and the number of 7S PFC usually disappeared totally in one to two transfer generations. Repeated attempts to reestablish antibody synthesis after the 7S PFC had disappeared by further transfers in the presence of antigen always failed (Table V).

The failure to carry out serial transfers of immune cells may be caused by elimination of the antigen-sensitive cells or, alternatively, by suppression of active antibody production even though antigen-sensitive cells were present.

A likely mechanism of suppression would be antibody feedback. This possibility was made unlikely by the finding that serum antibody production declined in parallel with the number of 7S PFC during the last transfer generations (Fig. 2). It seems more likely that antigen-sensitive cells have a limited lifetime and/or a restricted capacity for multiplication, even if they are repeatedly stimulated by antigen. Furthermore, such cells do not seem to develop spontaneously at any greater rate, since renewed appearance of 7S PFC did not occur during several successive transfers in the presence of antigen.

DISCUSSION

The findings that a large and sometimes major part of the spleen cells in irradiated, nonsensitized recipients of antigen-stimulated immune cells consists of specifically 7S-producing cells, indicate that normal homeostatic mechanisms limiting the number of antibody-producing cells have been abrogated or suppressed by the experimental procedure. Most likely one cause of the excessive multiplication of 7S PFC is the absence of antibody feedback, but other factors are probably also involved, such as an increased possibility for cellular multiplication in irradiated hosts. However, the absence of antibody feedback appears to be the dominating cause, since analogous transfers into irradiated and immunized hosts led to a marked suppression of 7S synthesis. Thus, irradiation as such does not favor the excessive multiplication of 7S PFC. This is also shown by the low number of 19S PFC found in the spleens of irradiated recipients given antigen-stimulated nonimmune lymphoid cells (10). The results also demonstrate that there are no inherent limitations of the proliferative ability of the immunocompetent cells themselves, which have the capacity to multiply vigorously under appropriate conditions.

Since the spleens of some animals were found to consist almost entirely of 7S PFC, it seems likely that the agar plaque technique is capable of detecting all 7S antibody–producing cells. Since puromycin abolished the PFC, it could be excluded that adsorbed antibodies onto cells or cellular debris were responsible for the PFC.

Antibody suppression appears to be an efficient mechanism to suppress multiplication and/or differentiation of antibody–producing cells, but may also act to select cells with high affinity for the antigen. The antibodies appear to suppress by combining with the antigenic determinants, which are necessary for stimulation of the antigen-sensitive cells. It is irrelevant to the principle of antibody suppression in what form the antigenic determinants are presented to the responding cells and whether intermediate steps of antigen localization and catabolism are involved. The selective activity of antibody suppression would operate through competition between immune cells and serum antibodies for the antigen. Presumably the antigen-sensitive cells are equipped with specific receptors of antibody nature. The outcome of the competition

would be determined by quantitative factors and by the relative affinity of the antibody receptors. Cells with a high affinity for the antigen would have a greater probability of reacting with the antigen and being stimulated to antibody synthesis. These antibodies would suppress cells with a lower affinity. Thus, antibody suppression would select cells producing high affinity antibody. Experimental findings concerning the increase of antibody affinity with time are in agreement with this postulate (16). It would be expected that the transfer of immune cells and antigen into recipients lacking suppressive antibodies would give rise to a more heterogenous antibody population, at least initially, since selection against low affinity cells would be diminished. However, by repeated transfers of immune cell population it is likely that a selective pressure is created. Although antibody suppression is weak in this system it probably exists, since high titers of antibody are eventually formed. The outcome of this with regard to antibody characteristics is difficult to predict, but will be a subject of further studies.

If it is assumed that the 7S PFC are derived by division it would be possible to calculate the number of divisions involved. By necessity such calculations are uncertain, since several unsupported assumptions are needed. The mean proportion of antibody-producing cells is about 0.05% in the original inoculum, corresponding to 5×10^3 inoculated PFC. It will be assumed that the number of antigen-sensitive cells is the same, although it cannot be stated whether they are identical to or different from the PFC. Furthermore, only 1% (50 cells) or less of the injected cells appear to settle down in the spleen (reference 15 and Table I) and the number of 7S PFC found 7 days later is 10^6. Thus, if the cells developed by division they multiplied 14 times. The mean generation time would be around 12 hr. The actual generation time found in the experiments documented in Fig. 1 was 9.6 hr between days 4 and 7. By extrapolation to 0 time it could be shown that this corresponded to one precursor cell. In the present calculation 50 precursor cells were postulated and accordingly the generation time was increased to 12 hr. If it is assumed that the generation time determined experimentally (9.6 hr) is correct, it follows that either there is only one precursor or, if 50 precursors are available, that there is a lag period before multiplication starts. Since the number of antigen-sensitive cells is likely to exceed one (15, 17) it is assumed that the value 50 is correct. If so, there is a lag period of 2 days before the development of 7S PFC. Thus, both the generation time and the lag period for 7S PFC in this transfer system is analogous to the values obtained for 19S cells in the intact mouse. In each of the earlier transfers the mean number of 7S PFC recovered per spleen was around 10^6 cells after the inoculation of about 10^7 spleen cells containing approximately 5×10^5 PFC as a mean. From the above assumptions it follows that the antigen-sensitive cells divided 38 times during the first four passages. Thus, one antigen-sensitive precursor would have the potential to give rise to nearly 10^{12} antibody-producing cells.

SUMMARY

Transfer of spleen cells from mice immunized against sheep red blood cells (SRBC) into irradiated (600 R) nonimmune, syngeneic mice in the presence of antigen resulted in excessive cellular 7S production 7 days later. The number of 7S plaque-forming cells usually exceeded 10^6 per spleen and the mean proportion varied between 1 and 70%. In occasional animals all spleen cells were producing antibodies to SRBC. Serum antibody synthesis was also excessively increased, the titers in agglutination after 2-ME treatment and in hemolysis varying between 2^{15} and 2^{25}. The generation time of the 7S PFC was found to be 9.6 hr in the secondary hosts.

It seemed possible that the excessive production of 7S PFC and antibodies in the irradiated nonimmune recipients was caused by the absence of feedback inhibition of the immune response by antibody, a mechanism which would normally function to restrict antibody synthesis. This conclusion was strengthened by the demonstration that transfer of antigen-stimulated immune cells into actively or passively immunized irradiated recipients resulted in a marked suppression of cellular 7S synthesis. Serial transfers of antigen-stimulated immune cell populations in irradiated hosts resulted in an equally high number of 7S PFC during the first four transfer generations. However, after the fifth to seventh transfer generation the number of 7S PFC rapidly declined and disappeared within one to three passages. Serum antibodies and 7S PFC declined in parallel during the last transfer generations. Further passages of antigen-stimulated spleen cells lacking 7S PFC did not lead to reappearance of PFC. Thus, antigen-sensitive cells have a limited lifespan and/or multiplication capacity. From the hypothesis that the 7S PFC developed by division from antigen-sensitive precursors it was calculated that 38–40 divisions occurred. Thus, one antigen-sensitive precursor has the potential to give rise to 10^{12} 7S PFC.

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