Hepatic and Splenic Phagocytosis of Isophilic and Heterophilic Antigens in Immunized Rats[1] (34675)

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Immunization of rats with heterophilic antigen (HA) interfered with immune responses to subsequent challenge of the animals with sheep red cells (SRC); formation of isophilic antibodies was almost completely suppressed (1, 2). A single exposure to HA of rats, 2–13 days old, significantly depressed levels of isophilic antibodies when SRC were injected into animals 10–20 weeks later (3). On the basis of several observations, cellular rather than humoral mechanisms appeared to be responsible for these phenomena. In order to obtain more insight into these cellular events, we compared phagocytosis of ^{51}Cr-labeled antigens (HA and SRC) in control rats and animals preimmunized with either HA or SRC. A preliminary report of this study has been presented (4).

Methods and Materials. Animals. Fischer-344 rats were obtained from Microbiological Associates (Walkersville, Maryland) or bred in our laboratories.

Antigens. SRC were obtained within the week of use from Clinical Laboratories Supplies, Skokie, Illinois. HA consisted of suspensions of boiled homogenate of guinea pig kidney (GPK), purchased from Mount Sinai Medical Research Foundation, Chicago, Illinois. For radiolabeling of HA, homogenates of GPK were prepared in our laboratory.

Radiolabeling of antigens. SRC were labeled with ^{51}Cr within 3 hr of use according to methods described previously (5, 6). For preparation of ^{51}Cr-labeled HA, a guinea pig was killed 1 day prior to expected use; the kidneys were immediately removed, placed in ice-cold saline, and homogenized. Red cells were removed from the homogenate by repeated centrifugation. To each 1 ml of packed homogenate, 0.5 ml of sodium radiochromate (Chromitope sodium, E.R. Squibb & Sons, New York) containing approximately 70 μCi was added, and the mixture was incubated at room temperature for 30 min. After three washings with saline, the homogenate was gently boiled for 30 min, and again washed three times with saline. The preparation was considered suitable for injection if the supernatant of the final washing contained only traces of radioactivity. On the day of use, a 7% suspension in saline of the radiolabeled homogenate was prepared.

Experimental designs. (a) Adult rats were divided into control and experimental groups. Blood samples were collected from the tail vein of each animal. Rats of experimental groups were injected intraperitoneally with 0.5 ml/100 g of body weight of a 10% suspension in saline of GPK (HA) or of a 10% suspension in saline of SRC. Rats of the control group were injected with the same amount of saline. Nine to 12 injections were given within 2–3 weeks. Three days after the last injection, another blood sample was collected from the tail vein. One day later, all rats of a particular experiment were injected intraperitoneally either with 0.5 ml/100 g of body weight of a 10% suspension in saline of ^{51}Cr-SRC or with the same amount of a 7% suspension in saline of ^{51}Cr-HA.

(b) Newborn rats, 1–3 days old, were injected intraperitoneally with 0.1 ml of a 10% suspension in saline of HA or SRC.

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Littermates, serving as controls, received 0.1 ml of saline. After 10–13 weeks, blood samples were collected from the tail veins of control and experimental rats, and 1 day later, all animals of a particular experiment were injected with ^{51}Cr-labeled antigen, as described for adult rats.

In some experiments, after injection of radiolabeled antigens, rats were placed in metabolic cages for collection of fractional urine samples. In all experiments, rats of control and experimental groups were anesthetized with ether 24 hr after injection of radiolabeled antigen and exsanguinated by cardiac puncture. Livers and spleens were removed, weighed, and placed into plastic tubes for radioassay.

Estimation of phagocytosis. Aliquots of the suspensions of the radiolabeled antigen, used for injection, were radioassayed in a scintillation well counter (RIDL, model 60-2, 11-1 well, 1.75 × 2-in. crystal), in parallel with the organs, urine, and blood samples. Radioactivity recovered in liver and spleen was expressed as percentage of injected radioactivity per organ and per unit of weight, and these values served as indicators of hepatic and splenic phagocytosis (4–6). Radioactivity found in blood was calculated as percentage of injected radioactivity in the whole blood volume, estimated as 13% of body weight.

Determination of antibodies. Agglutinins and hemolysins for SRC were assayed in serums of blood collected from each animal (a) prior to immunization; (b) 24 hr before, and (c) 24 hr after injection of radiolabeled antigen. Methods for determination of antibodies have been described previously (2).

Evaluation of results. Mean values and their standard deviations were calculated for phagocytic uptakes in liver and spleen, radiolabel found in blood and urine, and antibody levels, as determined in rats of each group. Statistical significance of differences between groups was ascertained by means of Student's *t* test.

Results. Since size of liver and spleen, expressed as percentage of body weight, did not differ in rats of control and experimental

TABLE I. Uptake of ^{51}Cr-SRC in Adult Rats Immunized with HA or SRC.[a,d]

Exp. no.	Sex	Age (months)	Group	No. of rats	Injected radioactivity (% ± SD) in						Hemolysin (H_{50} U/ml ± SD)		
					Liver		Spleen		Blood (total vol)	Urine (24 hr)	Before ^{51}Cr-SRC (total)	After ^{51}Cr-SRC (total)	Isohemolysin
					(whole)	(g)	(whole)	(100 mg)					
I	F	12–13	Control	8	11.1 ± 3.8	1.9 ± 0.6	1.4 ± 0.2	0.4 ± 0.1	14.0 ± 4.6	23.0 ± 12.1	—	—	—
			HA	11	4.7 ± 1.4[b]	0.6 ± 0.1[b]	0.8 ± 0.1[b]	0.2 ± 0.1[b]	13.3 ± 3.1	28.2 ± 25.7	11 ± 8	19 ± 36	—
			SRC	7	3.7 ± 1.0[b]	0.5 ± 0.1[b]	0.8 ± 0.4[b]	0.2 ± 0.1[b]	9.9 ± 13.3	22.8 ± 18.1	246 ± 67	405 ± 89	91 ±30
II	M	4–8	Control	6	8.4 ± 2.5	0.9 ± 0.4	1.0 ± 0.2	0.2 ± 0.1	11.2 ± 2.0	ND[c]	—	—	—
			HA	9	3.5 ± 0.3[b]	0.5 ± 0.1[b]	0.6 ± 0.1[b]	0.1 ± 0.2	11.8 ± 1.3	ND	28 ± 29	87 ±104	—
			SRC	5	4.0 ± 1.2[b]	0.5 ± 0.2	0.9 ± 0.3	0.2 ± 0.1	10.8 ± 2.3	ND	78 ± 59	175 ±161	15 ±17
III	M	3	Control	3	5.3 ± 0.9	0.6 ± 0.2	0.7 ± 0.5	0.2 ± 0.1	ND	29.3 ± 4.7	—	—	—
			HA	8	4.3 ± 0.5[c]	0.5 ± 0.1	1.0 ± 0.2	0.2 ± 0.1	ND	19.3 ± 7.0	28 ± 48	23 ± 46	—
			SRC	4	3.8 ± 0.3[c]	0.4 ± 0.1	0.8 ± 0.1	0.1 ± 0.0[c]	ND	26.4 ± 0.4	477 ±102	442 ±189	35 ±19

[a] Comparison between control and experimental groups: [b] $p = <0.01$; [c] $p = <0.05 > 0.01$.
[d] Brace indicates comparison between HA and SRC groups.
[e] ND = not done.

groups of any experiment, these values have been omitted from tabulation. This applies also to antibody levels in serum samples collected prior to immunization of rats; in no instance did they exceed one 50% unit of hemolysin (H_{50} U) per milliliter or an agglutinin titer of 1:2, as measured by twofold serial dilutions.

Table I presents experiments in which adult rats were injected with ^{51}Cr-SRC after immunization with HA or SRC. In Expt. I, hepatic and splenic uptakes of radiolabel were significantly reduced in rats previously subjected to immunization with HA or SRC. Rats preimmunized with SRC showed markedly higher levels of antibodies than rats immunized with HA. Control rats, not previously immunized, formed no measurable antibodies, as was to be expected from the 1-day interval between administration of the radiolabeled antigen and termination of the experiment. The decreased localization of radiolabel in liver and spleen of experimental rats was not correlated with changes in blood concentration or urinary excretion of the radiolabel. Results of Expts. II and III, in which male rats of younger ages were used, were comparable to those of Expt. I, but depression of splenic phagocytosis in experimental groups was less apparent.

Table II summarizes experiments in which uptake of ^{51}Cr-HA was compared in adult control rats and animals preimmunized with HA or SRC. In Expts. I and II, rats preimmunized with HA showed a trend toward increased hepatic and splenic uptakes of radiolabel, and in Expt. III an increased splenic uptake was seen, but the differences lacked statistical significance. Increased splenic uptakes of ^{51}Cr-HA, seen in animals preimmunized with SRC (Expts. II and III), were also insignificant. Levels of hemolysin in preimmunized rats, before and after injection of ^{51}Cr-HA, were similar to those found in comparable animals given ^{51}Cr-SRC (Table I). Blood levels of radiolabel were significantly depressed in rats preimmunized with HA (Expts. I and III) and in rats preimmunized with SRC (Expts. II and III).

Table III presents data obtained from two

TABLE II. Uptake of ^{51}Cr-HA in Adult Rats Immunized with HA or SRC.[a,d]

Exp. no.	Sex	Age (months)	Group	No. of rats	Injected radioactivity (% ± SD) in: Liver (whole)	Liver (g)	Spleen (whole)	Spleen (100 mg)	Blood (total vol)	Urine (24 hr)	Hemolysin (H_{50} U/ml ± SD): Before ^{51}Cr-HA (total)	After ^{51}Cr-HA (total)		Isophilic
I	F	13	Control	5	6.4 ± 5.3	0.8 ± 0.7	0.9 ± 1.0	0.2 ± 0.2	8.0 ± 1.9	4.8 ± 1.9	ND[c]	—		—
			HA	6	8.8 ± 4.3	1.2 ± 0.9	1.6 ± 0.8	0.3 ± 0.2	5.0 ± 1.8[c]	4.9 ± 0.7	ND	35 ± 29	[b]	—
			SRC	3	5.9 ± 1.3	0.8 ± 0.2	0.9 ± 0.6	0.1 ± 0.1	7.8 ± 1.9	5.8 ± 1.0	ND	255 ± 134		65 ± 48
II	M	6	Control	6	9.8 ± 1.4	1.1 ± 0.2	1.1 ± 1.1	0.2 ± 0.2	13.5 ± 2.1	ND	—	—		—
			HA	5	12.9 ± 3.3	1.4 ± 0.4	1.7 ± 0.7	0.3 ± 0.2	10.8 ± 5.6	ND	23 ± 22	21 ± 19	[c]	—
			SRC	6	6.8 ± 0.9	0.7 ± 0.1[b]	1.5 ± 0.5	0.3 ± 0.1	9.4 ± 2.3[c]	ND	214 ± 106	143 ± 82		53 ± 41
III	M	4–8	Control	3	6.7 ± 2.5	0.6 ± 0.3	1.5 ± 0.4	0.2 ± 0.2	8.1 ± 0.6	ND	—	—		—
			HA	7	5.6 ± 1.9	0.7 ± 0.2	2.2 ± 1.3	0.4 ± 0.3	4.7 ± 2.2[c]	ND	16 ± 13	32 ± 35	[c]	—
			SRC	3	6.2 ± 0.5	0.7 ± 0.1	1.9 ± 0.4	0.3 ± 0.1	4.3 ± 1.0[b]	ND	115 ± 39	233 ± 231		67 ± 75

[a] Comparison between control and experimental groups: [b] $p = <0.01$; [c] $p = <0.05 > 0.01$.
[d] Brace indicates comparison between HA and SRC groups.
ND = not done.

TABLE III. Uptake of ^{51}Cr-SRC or ^{51}Cr-HA in Rats Injected Neonatally with HA or SRC.[a]

Group	^{51}Cr-labeled antigen	No. of rats	Injected radioactivity (% ± SD) in Liver (whole)	Liver (g)	Spleen (whole)	Spleen (100 mg)	Blood (total vol)	Urine (24 hr)
Control	^{51}Cr-SRC	18	12.4 ± 5.3	2.0 ± 0.3	1.2 ± 0.4	0.3 ± 0.1	8.7 ± 2.8	25.7 ± 12.2
HA		10	5.4 ± 1.2[b]	0.8 ± 0.1[c]	0.7 ± 0.1[b]	0.2 ± 0.03[c]	11.2 ± 3.2[c]	31.5 ± 10.9
SRC		17	10.3 ± 4.8	1.7 ± 0.9	1.1 ± 0.4	0.3 ± 0.1	8.7 ± 4.3	26.0 ± 8.5
Control	^{51}Cr-HA	11	11.8 ± 7.1	1.7 ± 1.8	1.2 ± 0.8	0.3 ± 0.1	8.9 ± 3.6	5.5 ± 2.1
HA		7	8.0 ± 4.6	1.1 ± 0.7	1.4 ± 1.2	0.3 ± 0.2	7.4 ± 5.7	4.6 ± 0.7
SRC		8	12.2 ± 3.2	1.8 ± 0.7	1.6 ± 1.3	0.3 ± 0.2	9.5 ± 5.2	2.9 ± 0.8

[a] Comparison between control and experimental groups: [b] $p = <0.01$; [c] $p = <0.05 > 0.01$.

experiments in which 1–3-day-old littermates were injected with either saline, HA, or SRC, and challenged with ^{51}Cr-SRC or ^{51}Cr-HA at the age of 10–13 weeks. In rats treated neonatally with HA, deposition of ^{51}Cr-SRC in liver and spleen was significantly depressed, whereas rats treated neonatally with SRC exhibited uptakes comparable to those of saline-injected controls. A moderate, but significant, elevation of blood levels of radiolabel, was found in HA-treated rats. Samples of blood from rats neonatally given HA or SRC, collected 1 day before, and 1 day after the administration of ^{51}Cr-SRC, contained identical hemolysin levels of 5 H_{50} U/ml, except that 1 H_{50} U/ml of isophilic antibody was found in serums of rats injected neonatally with SRC.

When rats treated neonatally with either HA or SRC were injected with ^{51}Cr-HA at the age of 10–13 weeks, control and experimental groups did not differ from each other in hepatic and splenic localization, blood levels, and urinary excretion of radiolabel. One day prior to administration of radiolabeled antigen, serum antibody levels were 5 H_{50} U/ml in HA-treated rats and 9 H_{50} U/ml in SRC-treated rats. At the end of the experiment, serums of HA-treated rats had 4 H_{50} U/ml; SRC-treated rats had 18 ± 27 H_{50} U/ml, of which 3 ± 2 H_{50} U/ml were isophilic antibodies.

Discussion. When adult rats were immunized first with HA, their hepatic and splenic uptakes of radiolabeled SRC were significantly lower than those found in control rats. The only exception to this behavior was unchanged splenic phagocytosis of ^{51}Cr-SRC in one group of HA-treated rats (Table I, Expt. III). On the other hand, immunization with HA did not depress uptake of radiolabeled HA. Immunization of adult rats with SRC decreased splenic localization of ^{51}Cr-SRC in one, and hepatic localization in all, experiments. Exposure of newborn rats to HA or SRC had a more striking effect on uptake of antigen administered during adult life. Significant depression of hepatic and splenic phagocytosis occurred only when rats treated neonatally with HA were challenged with ^{51}Cr-SRC. By contrast, neonatal treatment with HA did not change the uptake of ^{51}Cr-HA, and neonatal administration of SRC failed to interfere with subsequent localization of either antigen.

These observations closely parallel the previously reported inhibition of immune responses to SRC of rats immunized with HA during adult life (2) or treated with HA in the neonatal period (3). Although passive immunization with antibodies for SRC or for HA was also found to inhibit immune responses to subsequently injected SRC (2), the reduced formation of antibodies for, and depressed phagocytosis of, SRC resulting from pretreatment with HA, cannot be attributed primarily to presence of circulating antibodies. In rats exposed to HA neonatally, circulating antibodies were absent, or minimal, at the time when they were immunized with, or tested for uptake of, SRC. Rats exposed neonatally to SRC had similar low

levels of hemolysin as did rats treated with HA, but uptake of ^{51}Cr-SRC was depressed only after exposure to HA. Adult rats immunized with SRC had significantly larger amounts of hemolysin than did adult rats immunized with HA, prior to administration of ^{51}Cr-SRC. Injection of ^{51}Cr-SRC into mice previously immunized with SRC resulted in accelerated and increased elimination of radiolabel in urine and feces and decreased uptake in liver and spleen (unpublished data). As shown in Tables I and II we did not observe any differences in urinary excretion of radiolabel between control and experimental groups, but we did not determine fecal excretion. The possibility cannot be excluded that the reduced phagocytic uptake of ^{51}Cr-SRC in adult rats immunized with SRC reflected rapid destruction of substrate prior to its reaching liver and spleen. However, this mechanism cannot account for the results obtained after immunization with HA, particularly of rats treated neonatally. Finally, antibody levels in serums of experimental rats, determined before and after administration of ^{51}Cr-SRC (Table I) did not differ essentially from those found before and after injection of ^{51}Cr-HA (Table II), but localization of the latter antigen was not depressed.

Interpreting the interference with immune response of rats to SRC after immunization with HA (2), we proposed that this phenomenon was mediated by "cellular diversion of antigen." Basically, this hypothesis includes three assumptions: (I) exposure to HA imprints suitable cells with receptors for HA; (II) imprinted cells produce clones, consisting of large numbers of cells, carrying HA-specific receptors; (III) subsequently introduced SRC, which carry sites of HA, as well as of isophilic antigen (IA), are diverted to HA-imprinted cells and thus deprived of contact with cells better equipped to process substrate containing both HA and IA.

While the particular type of cell responsible for this phenomenon could not be specified, it is generally accepted that particulate antigens are taken up by reticuloendothelial cells of liver and spleen (7). Hepatic phagocytosis has been implicated as an important step in processing of antigen (8). The role of the spleen in phagocytosis of antigen and synthesis of antibody has been convincingly demonstrated (9). Stimulation of reticuloendothelial function by various agents enhanced immune responses to particulate antigens (10). Peritoneal macrophages were shown to trigger antibody production in X-irradiated animals (11) and to initiate antibody formation in neonatal mice which were immunoincompetent unless so treated (12). Fishman (13) postulated that participation of macrophages in immune processes may involve: (i) scavenging; (ii) processing of antigen; and, possibly, (iii) synthesis of antibody.

Results of this study point to macrophages as being at least in part responsible for the inhibition of immune responses to SRC following immunization of rats with HA, inasmuch as their ability of trapping, and presumably processing, ^{51}Cr-SRC was impaired. This interpretation does not exclude the possible involvement of antibody synthesizing cells and/or circulating antibodies in the phenomenon described.

Summary. Hepatic and splenic uptakes of ^{51}Cr-labeled sheep red cells (SRC) were significantly depressed in adult rats which had previously been immunized with heterophilic antigen (HA, boiled homogenate of guinea pig kidney). Uptake of ^{51}Cr-labeled HA was not affected by preimmunization. Administration of HA to newborn rats significantly depressed uptake of ^{51}Cr-SRC, but not of ^{51}Cr-HA, given 10–13 weeks later. Administration of SRC to newborn rats failed to interfere with uptake of ^{51}Cr-SRC or ^{51}Cr-HA later in life. Since these observations parallel the reduced formation of antibodies for SRC in rats preimmunized with HA, the changes in phagocytosis and immune responses may be attributable to imprinting of numerous macrophages with receptors specific for HA. Subsequently introduced SRC may thus be diverted to HA-specific macrophages and be prevented from contact with macrophages better equipped to phagocytose and process this substrate containing both heterophilic and isophilic antigens.

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