PATHOGENETIC MECHANISMS IN EXPERIMENTAL IMMUNE FEVER

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Although fever is a well-known manifestation of some allergic or immune states, its pathogenesis in these situations remains obscure. Previous studies have demonstrated that animals immunized with protein antigens will readily respond with fever upon challenge with specific antigen (1, 2). In rabbits immunized and then challenged with human serum albumin (HSA), the febrile response has been shown to be proportional to both the antigen dose and titers of circulating antibody (2). Furthermore, the capacity to develop fever upon challenge can be passively transferred to normal rabbits only by using serum which contains measurable antibody (2, 3). These findings indicate that humoral antibodies participate in an essential manner in the etiology of this experimental fever. The present investigations were undertaken to determine how antigen and antibody interact within the host to produce fever. The results suggest that the formation of circulating antigen-antibody complexes probably play an important initial role in the pathogenesis of the febrile response.

Materials and Methods

New Zealand albino rabbits from the National Institutes of Health colony weighing from 1750 to 2800 g were used throughout the study. Details of their housing, the recording of temperatures, plotting of fever curves, immunization with HSA,[1] injections and bleedings, and precautions to exclude contaminating pyrogens were as reported previously (2). All serum pools were checked for sterility by culture on blood agar plates and in thioglycollate broth, and contaminated material was discarded. Sera were stored at either −20°C or −70°C until used.

Antibody Determinations.—Antibody titers were measured in serial two-fold dilutions of serum using the bentonite flocculation technique as previously described (2). Precipitin equivalence zones were determined by the addition of increasing amounts of ^{125}I-HSA[2] in 0.1 ml volumes to 0.5 or 1.0 ml quantities of whole immune serum. The ^{125}I-HSA-serum mixtures were then incubated at 37°C for 1 hr and 4°C for 2–4 days. At the conclusion of the incubation period, the antigen-antibody precipitates were removed by centrifugation (2000 *g* for 30 min at

[1] Courtland Laboratories, Los Angeles, Calif.

[2] Kindly prepared by W. H. Briner, Chief, Radiopharmaceutical Service, N.I.H., Bethesda, Md.

4°C) and washed twice with phosphate-buffered pH 7.4, 0.15 M saline (PBS). The activity of the ^{125}I-HSA in the precipitates was determined by counting in a NaI crystal well scintillation counter.[3] The total activity of the ^{125}I-HSA added at each concentration was determined by counting the protein precipitates formed after addition of 10% trichloroacetic acid (TCA). Thus, the per cent antigen precipitated by the antiserum at each concentration could be calculated by comparison with the total counts. The end point of the equivalence zone was indicated when the amount of antigen added was no longer totally precipitated by antibody. Standard precipitin-ring tests were also done on the supernatants remaining after removal by centrifugation of antigen-antibody precipitates to confirm the absence of free precipitating antibody at the equivalence point (4).

Passive Transfer Studies.—At intervals (5 min and 2 hr) after intravenous challenge with 25 mg of HSA, immunized and normal rabbits were exsanguinated under sterile conditions by cardiac puncture and the blood allowed to clot at room temperature for 1–1.5 hr before storing overnight at 4°C. The next day serum was separated by centrifugation and cultured. Sera from immune donors obtained 2 hr after HSA challenge (2-hr serum) were divided into two groups and pooled: (*a*) those that were from febrile donors (temperature > 0.3°C above base line) at the time of bleeding, and (*b*) those that were from afebrile donors. Sera from normal donor rabbits (all afebrile) were similarly pooled. The sera obtained 5 min after HSA challenge (5- min serum) from immune and normal donors were also pooled, cultured, and stored at 4°C until used. In several experiments in which blood was obtained 5 min after HSA challenge, heparin (10 units/ml) was added, the cells removed by centrifugation, and the plasma saved for demonstration of circulating pyrogens.

Assay for pyrogens in donor sera or plasma was performed by intravenous transfer into trained, normal, or endotoxin-tolerant recipient rabbits. Preliminary experiments indicated that greater than 20 ml volumes (approximately 10 ml/kg) were required to consistently demonstrate the presence of circulating pyrogens, so approximately 20 ml/kg volumes were used unless otherwise noted. All sera were incubated 15–30 min at 37°C prior to infusion.

Clearance of ^{125}I-HSA in Immune and Normal Rabbits.—25 mg of ^{125}I-HSA were administered to normal and immunized rabbits and serial blood samples were obtained. Serum was separated by centrifugation, and the total ^{125}I-HSA activity was measured in 50 or 100 μl aliquots by counting precipitates formed after the addition of 10% TCA. Values for serum volume were calculated in 10 normal rabbits by extrapolating the linear portion of the clearance curves to zero time and determining the volume of distribution of the ^{125}I-HSA initial activity. Expressed as a function of body weight, the mean value for serum volume was found to be 45 ml/kg. Using this latter figure, the body weight of the immunized rabbits and the radioactivity of the 25 mg dose of ^{125}I-HSA, initial or 100% values were determined for the immune serum aliquots. Subsequent values were then expressed as a per cent of this initial figure and clearance curves constructed.

The amount of HSA bound to antibody was determined using a modification of the Farr technique (5, 6). After dilution of either a 50 or 100 μl aliquot of serum in 1 ml of PBS plus 1 ml of borate buffer (pH 8.4), 2 ml of saturated ammonium sulfate (AS) were added to precipitate the globulins (final concentration of AS, 1/2 saturated). After incubation at 4°C for 30 min, the precipitated globulins were centrifuged in the cold at 2500 *g* for 30 min, washed once in half-saturated AS, and the precipitates counted. Using the per cent of the initial activity remaining at each time interval, a clearance curve for soluble antigen-antibody complexes was then constructed. The amount of ^{125}I-HSA bound to antibody was also calculated at each time interval as a per cent of the total (TCA-precipitable) activity.

Formation of Soluble Antigen-Antibody Complexes.—Immune precipitates were formed at

[3] Nuclear-Chicago Corp., Model No. 4223, Des Plaines, Ill.

equivalence by the addition of an appropriate amount of HSA to whole immune serum. The mixture was kept at room temperature or incubated at 37°C for 1 hr, then overnight at 4°C. Soluble complexes in antigen excess were formed the next morning by the addition of HSA directly to the immune serum containing the precipitates or to precipitates removed by centrifugation (2000 *g*), washed twice in PBS, and resuspended in normal serum. In some experiments, soluble complexes were formed without overnight incubation by the addition of excess antigen to whole immune serum. After shaking the mixtures at room temperature for 30 to 45 min, any remaining precipitates were removed by centrifugation (2000 *g* for 30 min at 4°C). The supernatants containing the soluble antigen-antibody complexes were then incubated at 37°C and injected intravenously into rabbits to test for pyrogenic activity. Using the technique described for determination of soluble complexes of antibody-bound ^{125}I-HSA, it was found that when 10 times the amount of antigen required to precipitate antibody at equivalence was added to precipitates, approximately 50% of the activity in the supernatant was bound to antibody as soluble complexes with the remainder consisting of free antigen. To insure that serum and antigen solutions were pyrogen free, separate equivalent amounts of each were administered to control rabbits.

Tolerance to Endotoxin.—Normal rabbits were made tolerant to the pyrogenic activity of *Escherichia coli* endotoxin by seven daily intravenous injections of 3 μg.[4]

Tolerance to Complexes.—Febrile unresponsiveness to soluble antigen-antibody complexes was produced by daily (five to six) intravenous infusions of 20 ml amounts of soluble complexes prepared in antigen excess at 10 times equivalence ("10 × complexes").

RESULTS

Circulating Serum Pyrogens.—

2-hr serum pyrogen: A pyrogen was demonstrated in the circulation of febrile immunized donors 2 hr after challenge with HSA by the production of fever in normal or endotoxin-tolerant recipient rabbits receiving passively transferred serum (Table I). This pyrogen was not found in the serum of immunized donors of comparable antibody titer nor in normal donors, both of whom had been challenged with HSA but remained afebrile (Fig. 1). In all cases, the fever curves were monophasic; after a latent period of 10 to 30 min, peak temperatures were reached by 60–80 min.

5-min serum pyrogen: The pyrogen demonstrable in the circulation of febrile immunized rabbits 2 hr after challenge with HSA appeared to have the characteristics of endogenous serum pyrogen (EP). This material is known to appear only during fever (7); therefore, to investigate this feature of the 2-hr pyrogen, serum was obtained from immunized donor rabbits 5 min after challenge with 25 mg of HSA and infused into normal recipients. Despite the absence of fever in the donor rabbits, a transferable pyrogen was also present at this time (Table I, Fig. 2). Like the 2-hr serum pyrogen, recipient fever curves were monophasic but had a latent period varying from 30 to 40 min and reached a peak by 80–150 min.

Clearance of HSA in Immunized and Normal Rabbits.—Further experiments were undertaken to elucidate the nature of this early appearing pyrogen and to

[4] Difco Laboratories, Detroit, Mich.

TABLE I

Pyrogenic Properties of Passively Transferred Serum from HSA-Immunized Rabbits after Challenge with 25 mg HSA

Donor	Recipient	No. of recipients	Time serum obtained	ΔT*	5-hr FI‡
				°C	cm^2
Immune	Normal	12	2 hr	0.81 ± 0.10§	10.5 ± 1.3
Normal	"	3	2 "	<0.2	0
Immune‖ (afebrile)	"	3	2 "	<0.2	0
Immune	Normal	6	2 "	0.67 ± 0.13¶	8.5 ± 2.1¶
Immune	Endotoxin-tolerant	8	2 "	0.59 ± 0.11¶	5.4 ± 1.2¶
Immune	Normal	8	5 min	0.95 ± 0.08	15.3 ± 4.5
Normal	"	4	5 "	<0.2	0

*ΔT is the maximum change in temperature above the base line in °C.

‡ FI is the fever index (see text) in cm^2 for 5 hr after challenge.

§ Mean ± standard error.

‖ The donor rabbits all had antibody titers (bentonite flocculation) of 1:32 but did not develop fever upon challenge with HSA.

¶ Differences are not significant (*t* test).

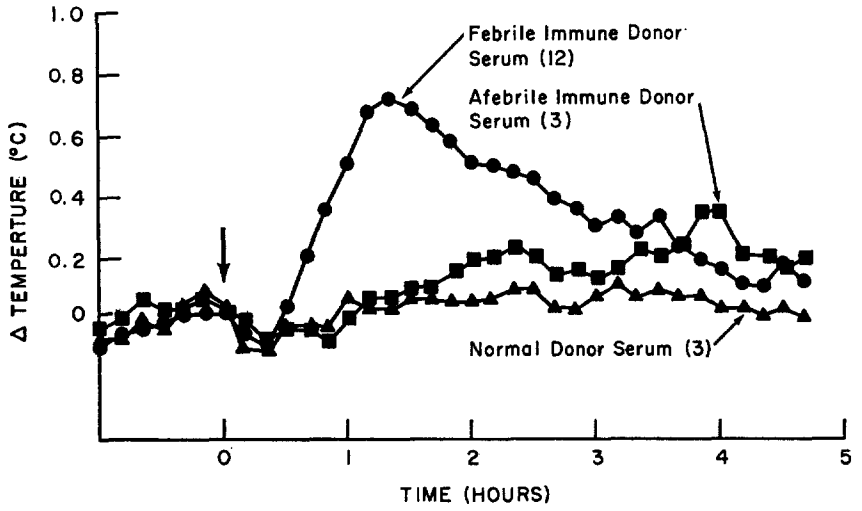

FIG. 1. Mean febrile responses of normal recipients (number of rabbits in parentheses) following the infusion at 0 time of 40 ml of sera obtained from normal donor rabbits (▲—▲), afebrile immune donor rabbits (■—■), and febrile immune donor rabbits (●—●) 2 hr after intravenous challenge with 25 mg of HSA.

attempt to separate it from the pyrogen circulating during fever. The clearance of intravenously administered HSA was examined in normal and immune rabbits. 25 mg doses of ^{125}I-HSA were given, and serum obtained at appropriate intervals thereafter was analyzed for the presence of total and antibody-bound HSA by the TCA and ammonium sulfate precipitation techniques.

Fig. 3 illustrates the mean febrile response of four immune rabbits of com-

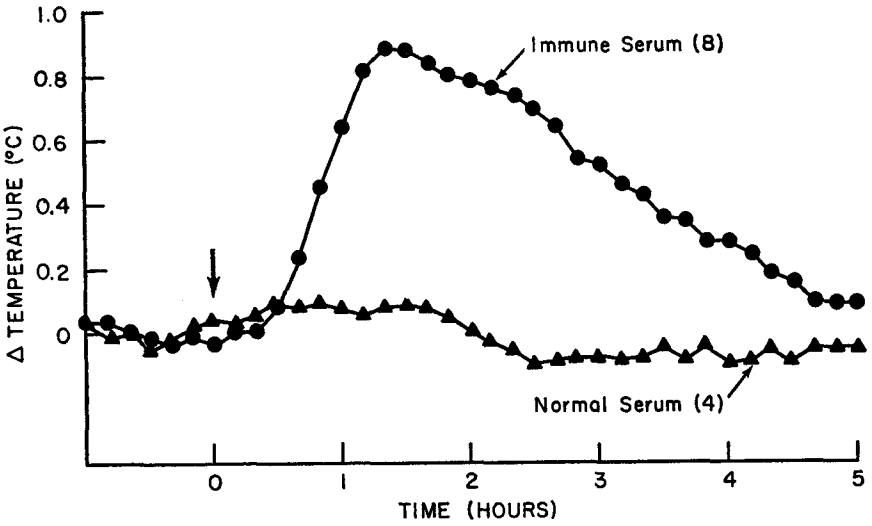

FIG. 2. Mean febrile responses of normal recipient rabbits (number of rabbits in parentheses) following the infusion at 0 time of 40 ml of sera obtained from normal donor rabbits (▲—▲) and immune donor rabbits (●—●) 5 min after the administration of 25 mg of HSA.

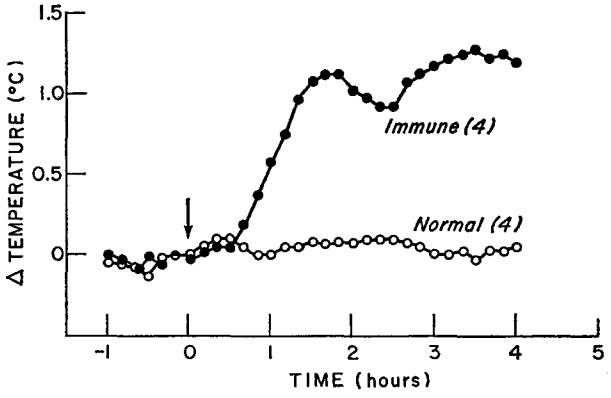

FIG. 3. Mean febrile responses of normal (○—○) and HSA immunized (●—●) rabbits (number of rabbits in parentheses) following the intravenous administration of 25 mg ^{125}I-HSA at 0 time.

parable antibody titers challenged with ^{125}I-HSA. In Fig. 4 the mean 4-hr clearance curves of total (immune) and antibody-bound (immune complexes) serum ^{125}I-HSA activity in the same immunized rabbits are compared to the

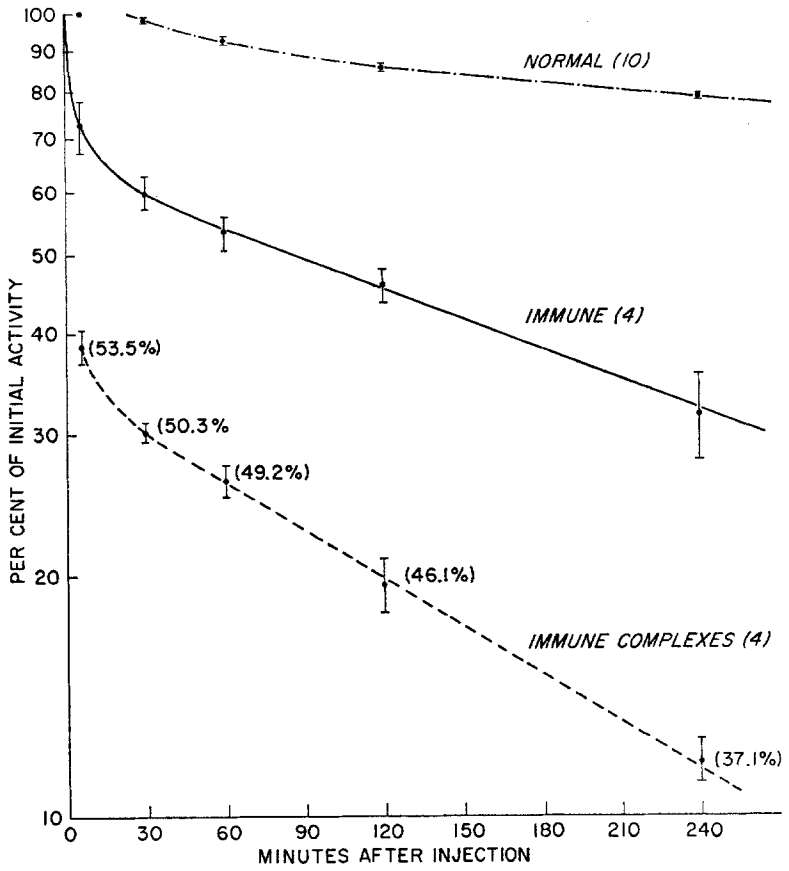

FIG. 4. Serum clearance of ^{125}I-HSA administered at 0 time to normal and HSA-immunized rabbits (number of rabbits in parentheses). Data is illustrated as the mean ± 1 SE for total (TCA-precipitable) ^{125}I-HSA activity in normal (.—.) and immunized rabbits (—) as well as clearance of soluble HSA-anti-HSA complexes (ammonium sulfate precipitable activity) in the same immunized rabbits (– – –). The percentages given in parentheses refer to the mean antibody bound radioactivity at each time interval.

mean clearance curve for total ^{125}I-HSA activity in 10 normal rabbits. Not shown are the values for ammonium sulfate precipitable ^{125}I-HSA activity in the normal rabbit sera which varied from 2 to 5 per cent of the total activity at all time intervals examined. The clearance of the antigen in the immune rabbits occurred in a biphasic manner with an early rapid phase followed by a more

prolonged slower phase. As indicated by the values in parentheses in Fig. 4, approximately 50% of the total ^{125}I-HSA activity in the immune serum at each time interval during the first 2 hr was bound to the globulin fraction and represented circulating soluble antigen-antibody complexes. When the amounts of circulating complexes in the immune sera, expressed as per cent of the total initial activity, were compared at 5 min to those at 2 hr, approximately 50% of the complexes had been cleared from the circulation between the two time intervals (range, 46.3–51.5%; mean, 50.1%) (Table II, Fig. 4). These findings suggested that the early pyrogenic factor might well be circulating antigen-antibody complexes.

TABLE II

*Clearance of Soluble ^{125}I-HSA–Anti-HSA Complexes in Immune Rabbits after Administration of ^{125}I-HSA**

Immune rabbit No.	Bentonite flocculation titer	Antibody-bound HSA remaining		Clearance of soluble complexes from 5 min to 2 hr
		5 min	2 hr	
		% initial activity‡		*% 5-min activity*§
1	1:512	49.5	24.0	51.5
2	1:128	37.6	16.5	56.0
3	1:128	37.2	19.9	46.5
4	1:128	29.9	17.2	46.3
Mean		38.5	19.4	50.1

*Dose, ^{125}I-HSA = 25 mg given intravenously.

‡ Amount of ^{125}I-HSA bound to serum globulin as determined by ammonium sulfate precipitation (see text) and expressed as per cent of total administered activity.

§ Calculated by expressing the difference between ammonium sulfate precipitable activity found at 5 min and 2 hr as per cent of the 5-min activity.

Pyrogenic Activity of Soluble Antigen-Antibody Complexes.—The pyrogenic properties of soluble antigen-antibody complexes prepared in vitro were next examined. In the initial experiments, soluble complexes were formed by adding excess antigen to immune precipitates in the original immune serum. After removal of the remaining precipitates by centrifugation, the supernatants containing the soluble complexes were infused into normal rabbits in approximately 10 ml/kg (20 ml) amounts. This produced characteristic febrile responses which, with rare exceptions, were preceded by a latent period of 30 to 50 min; were monophasic in type, reaching a peak temperature by 90 to 100 min after challenge; and lasted from 4 to 6 hr (Table III, Fig. 5). These fevers were similar to those exhibited by immunized animals challenged with HSA (2) or normal rabbits administered the 5-min serum. It was found that soluble complexes formed by the addition of 10 times the amount of antigen required to precipitate antibody at equivalence ("10 × complexes") elicited the most regular febrile

responses. As indicated in Table III, complexes produced by adding 21 or 3 × equivalence amounts of HSA caused generally smaller and more variable fevers.

When animals were made tolerant to the pyrogenic action of *E. coli* endotoxin,

TABLE III

Pyrogenic Properties of Soluble HSA–Anti-HSA Complexes Prepared In Vitro

Complexes: degree of antigen excess*	HSA bound at equivalence/ml antiserum	Serum‡	Recipient	No.	ΔT§	5-hr FI‖
	mg				*°C*	*cm²*
10 × equivalence	0.325	Immune	Normal	11	1.00 ± 0.09¶	16.7 ± 2.7¶
10 × equivalence	0.160	Normal	Normal	10	0.72 ± 0.12	11.1 ± 0.9
21 × equivalence	0.150	Normal	Normal	9	0.58 ± 0.08	9.2 ± 1.8
3 × equivalence	0.325	Normal	Normal	5	0.47 ± 0.15	7.2 ± 3.6
6–18 × equivalence	0.150–0.200	Immune	Normal	8	0.63 ± 0.10	8.3 ± 1.9
6–18 × equivalence	0.150–0.200	Immune	Endotoxin tolerant	7	0.50 ± 0.12	9.1 ± 3.3
None	—	Immune** supernatant	Normal	20	<0.2	0
None	—	Immune	Normal	8	<0.2	0
None	—	Normal‡‡ + HSA	Normal	9	<0.2	0

* Amount of HSA × amount bound at equivalence used to solubilize complexes.

‡ Complexes solubilized in either immune or normal serum and administered in a dose of 20 ml/rabbit.

§ ΔT is the maximum change in temperature above the base line in °C.

‖ FI is the fever index (see text) in cm^2 for 5 hr after challenge.

¶ Mean ± standard error.

** Immune serum remaining after antigen-antibody precipitates removed at equivalence.

‡‡ Normal rabbit serum containing the same amount of HSA used to solubilize complexes.

their febrile responses did not differ significantly from normal recipients given equivalent doses of soluble complexes in immune serum (Table III). Further confirmation that soluble complexes were the pyrogenic factor in the immune serum containing excess antigen was obtained by removing antigen-antibody precipitates from immune serum at equivalence and resolubilizing them in

normal serum with excess HSA. Such normal sera containing the soluble HSA-anti-HSA complexes were regularly pyrogenic when infused (20 ml doses) into recipient rabbits, whereas the immune serum supernatant remaining after removal of the precipitates was not (Table III).

Finally, it was observed that animals receiving complexes also exhibited frequent immediate reactions suggestive of acute anaphylaxis and characterized by agitation, struggling, nystagmus, cyanosis and, rarely, death within 2 to 3 min.

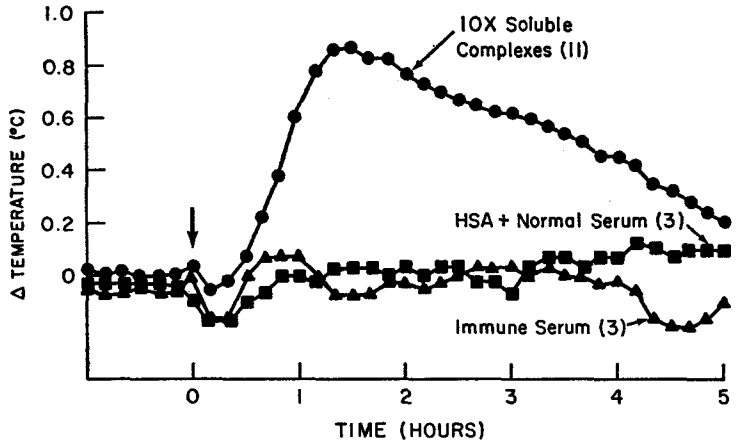

FIG. 5. Mean febrile responses of normal rabbits (number of rabbits in parentheses) following the infusion at 0 time of 10 ml/kg of either immune serum containing soluble complexes prepared at 10 × equivalence (●—●), normal serum containing an equal amount of HSA (■—■), or immune serum alone (▲—▲).

Febrile Tolerance to Antigen-Antibody Complexes.—Having demonstrated that soluble antigen-antibody complexes prepared in vitro were pyrogenic, the next series of experiments was designed to produce a state of febrile unresponsiveness (tolerance) to complexes. These experiments were performed in order to learn if fever caused by in vivo formed complexes in test sera could be separated from that due to endogenous pyrogen. As shown in Fig. 6 and summarized in Table IV, normal rabbits receiving daily infusions of 10 × soluble complexes in immune serum showed diminished febrile responses by 24 hr after the initial dose. Characteristically, on the second day the febrile responses were abolished almost entirely, with a tendency to rebound somewhat on day 3. This was followed again by minimal, if any, responses on days 4–6. In addition to reduction in febrile responses with succeeding doses, there was a concomitant decrease in the anaphylactic type responses. Complex-tolerant rabbits responded normally to *E. coli* endotoxin (Table IV), indicating that endotoxin contamination was not responsible for the tolerant state.

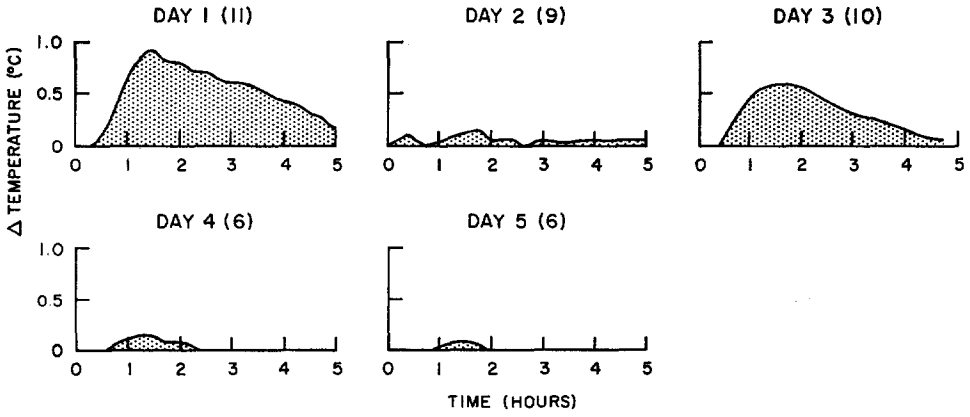

FIG. 6. Mean febrile responses of normal rabbits (number in parentheses) given a daily intravenous injection of 10 ml/kg of immune serum containing soluble antigen-antibody complexes prepared at 10 × equivalence. The figures demonstrate the development of pyrogenic tolerance.

TABLE IV

Responses of Complex-Tolerant Rabbits to Various Pyrogens

Test material	Recipient	No.	ΔT*	5-hr FI‡	2-hr FI§
			°C	cm^2	cm^2
HSA–anti-HSA soluble (10 ×) complexes‖	Normal (day 1)	14	1.02 ± 0.07¶	16.9 ± 2.1¶	—
	Complex tolerant (day 4)	11	0.25 ± 0.08	1.9 ± 0.8	—
2 hr serum	Complex tolerant (day 4–5)	8	0.54 ± 0.04	3.4 ± 0.3	3.4 ± 0.3
" "	Normal	6	0.55 ± 0.08	7.7 ± 2.0	4.6 ± 0.6
5 min serum	Complex tolerant (day 4)	10	0.22 ± 0.06	2.4 ± 2.3	—
" "	Normal	9	0.77 ± 0.15	11.9 ± 1.0	—
0.1 μg endotoxin	Complex tolerant (day 6)	11	1.75 ± 0.08	30.3 ± 2.4	—
" "	Normal	14	1.77 ± 0.08	33.5 ± 1.9	—

*ΔT is the maximum change in temperature above the base line in °C.

‡ FI is the fever index (see text) in cm^2 for 5 hr after challenge.

§ The fever index (see text) in cm^2 for 2 hours after challenge.

‖ Complexes solubilized in immune serum in 10 × antigen excess (0.350–0.400 mg HSA bound at equivalence/ml antiserum) and administered in 20 ml doses.

¶ Mean ± standard error.

Febrile Reactivity of Complex-Tolerant Rabbits to 5-min and 2-hr serum.—After the induction of febrile tolerance to soluble complexes by three or four daily infusions, animals were given either 2-hr serum or 5-min serum or plasma (in 40 ml quantities) obtained from HSA-challenged immune donor rabbits in order to separate pyrogenicity due to complexes from that due to endogenous pyrogen. As shown in Fig. 7 and summarized in Table IV, the 2-hr serum evoked

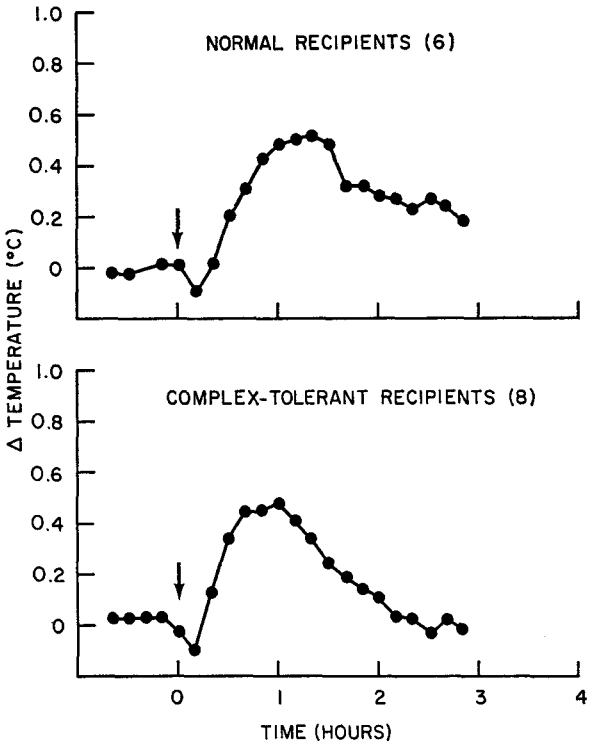

FIG. 7. Mean febrile responses of six normal and eight complex-tolerant rabbits administered 40 ml of serum obtained from febrile immunized donors 2 hr after challenge with 25 mg HSA (see text).

similar temperature rises in both normal and complex-tolerant rabbits. On the other hand, the pyrogenic activity of the 5-min serum was significantly reduced in complex-tolerant animals when compared to their normal counterparts (Table IV, Fig. 8). These results strongly suggested that the early-appearing circulating pyrogen consisted of soluble antigen-antibody complexes, and the later appearing factor was indeed endogenous pyrogen. The fact that the fevers were somewhat less prolonged in the tolerant recipients given 2-hr serum as demonstrated when 2-hr and 5-hr fever indexes (FI's) are compared (Table IV) is compatible with the persistence of some soluble complexes in the 2-hr serum.

DISCUSSION

In some patients undergoing febrile transfusion reactions, circulating platelet and leukagglutinins have been shown to play a causal role (8). In addition, fever regularly accompanies experimentally induced or naturally occurring immune hemolysis, which involves the interaction of hemolysins with foreign

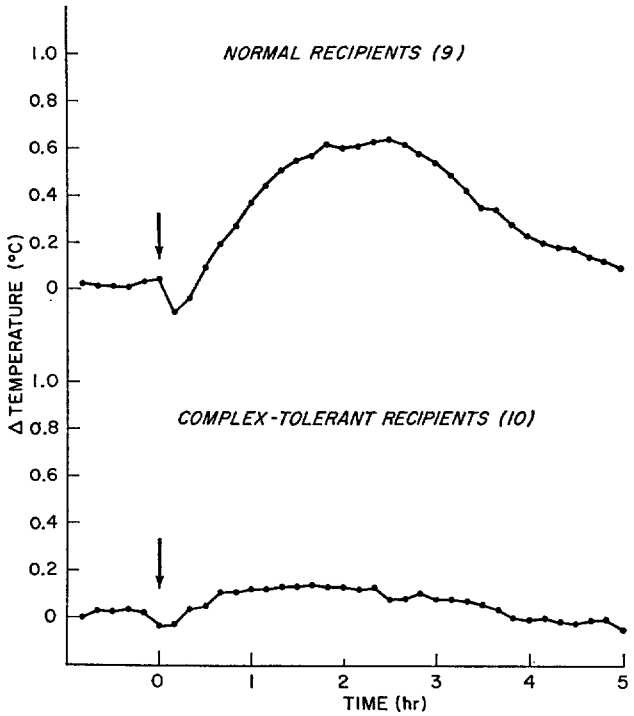

FIG. 8. Mean febrile responses of 9 normal and 10 complex-tolerant rabbits following infusion of 40 ml of serum or plasma obtained from immunized donors 5 min after challenge with 25 mg HSA (see text).

RBC's (9). In the initial studies of Farr and his associates with rabbits given BSA, the importance of the presence of circulating antibody to the subsequent development of fever on challenge with a protein antigen was demonstrated by passive transfer experiments (3). These observations were expanded by Mott and Wolff using HSA-immunized rabbits when a quantitative relationship between febrile response, antibody titer, and antigen dose was established (2). Thus, it seemed apparent that the interaction of antigen and circulating antibody in the host resulted in fever in these clinical and experimental conditions. The actual mechanism whereby fever developed in these situations remained to be defined.

A considerable body of evidence exists that many fevers are mediated by an EP released into the circulation from host leukocytes after interaction with exogenous agents such as endotoxin (10). Accordingly, the initial aim of the current studies was to see if the interaction of circulating antibody with intravenously administered antigen resulted in the appearance of EP. A transferable pyrogen with biological characteristics consistent with EP (i.e., monophasic fever, short latent period, and fever production in normal and endotoxin-tolerant rabbits) was demonstrated in sera obtained from febrile immune donors However, when sera were obtained from immune donors challenged with HSA prior to the development of fever, this also contained a pyrogen as demonstrated by infusion into normal recipients. Since EP is known to appear only at the time of fever (7), this finding suggested that some other early appearing circulating pyrogenic factor was also present in immunized donors challenged with antigen.

By using HSA-labeled with ^{125}I and a modification of the Farr technique to detect antibody-bound HSA, it was determined that a considerable portion of the administered antigen appeared in the circulation in combination with antibody as soluble antigen-antibody complexes. Evidence was then obtained that the early appearing transferable pyrogen represented circulating soluble antigen-antibody complexes. First, such complexes prepared in antigen excess in vitro were found to be regularly pyrogenic when administered to either normal or endotoxin-tolerant recipients. Second, by administering daily doses of complexes to rabbits, febrile responses to subsequent infusions could be abolished, and when such "complex-tolerant" rabbits were given sera obtained 5 min after challenge of immune rabbits with antigen, their responses were significantly less than normal controls. On the other hand, the infusion of sera obtained from febrile immune donors 2 hr after challenge with antigen caused equal temperature rises in normal and complex-tolerant recipients, indicating that this pyrogen, most likely EP, differed from the early appearing factor. The fact that the temperature responses in the normal rabbits were somewhat more prolonged than in the complex-tolerant rabbits given 2-hr serum can be explained by the presence of both soluble complexes and EP in the 2-hr serum acting as pyrogenic factors.

In relation to these findings, it is of interest that antigen-antibody complexes are known to be capable of initiating a variety of biological phenomena in various species, including anaphylaxis (11–14), serum sickness (15), nephritis (16, 17), and Arthus reactions (18). Their role in this latter reaction is particularly significant in light of the present studies, since the Arthus reaction is known to require the intimate participation of host leukocytes for its full elaboration (19). In vitro studies have shown that antigen-antibody complexes are chemotactic to (20) and can be phagocytized by leukocytes (21), with resultant degranulation and release of certain permeability factors (22). It seems reasonable, therefore, to speculate that complexes formed in vivo may activate host leukocytes to release leukocytic pyrogen, thereby inducing fever.

One the basis of our findings, therefore, it may be hypothesized that the fever produced by the interaction of circulating antigens with antibodies follows a biphasic reaction. The first phase consists of the formation of antigen-antibody complexes, both soluble and insoluble, and the second phase consists of the elaboration of an endogenous pyrogen, presumably from host leukocytes which have been activated after ingestion of antigen-antibody complexes. This hypothesis might be summarized as follows:

$$\text{Ag} + \text{Ab} \rightarrow \text{Ag} - \text{Ab complexes} + \text{WBC} \rightarrow \text{EP}$$

In this regard, the failure of HSA alone to release pyrogen from leukocytes obtained from sensitized donors (23)[5] might support the concept of the requirement for complex formation between antigen and serum antibodies in the first phase of the pyrogenic reaction. We are currently examining in detail the ability of antigen-antibody complexes to release pyrogen from normal and sensitized leukocytes, the results of which will form the basis for a later report.

SUMMARY

When rabbits sensitized to human serum albumin (HSA) are challenged intravenously with specific antigen, fever develops and two transferable pyrogens can be demonstrated in the circulation. The first appears prior to the development of fever and has properties consistent with soluble antigen-antibody complexes. These have been shown to be pyrogenic when prepared in vitro and to produce a state of febrile tolerance when repeatedly administered. The second pyrogen, demonstrable during fever in donor rabbits, appears to be similar to endogenous pyrogen described in other experimental fevers. It is postulated that the formation of antigen-antibody complexes constitutes an important initial phase of the febrile reaction in this type of immune fever.

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