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Research Article

THE PASSIVE TRANSFER OF SENSITIVITY TO ANTIGEN-INDUCED FEVER *

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The febrile response of immunized rabbits to bovine serum albumin (BSA) has been shown to be similar in many respects to the fever observed following the injection of bacterial endotoxin into normal rabbits and of old tuberculin into tuberculin-sensitive rabbits (1, 2).

The similarities between fever attributed to antigen and the fever induced by endotoxin have led to the suggestion that endotoxin is pyrogenic because it is itself an antigen. It has been postulated that sensitization to endotoxin results from the constant antigenic stimulus of endotoxin in the gram-negative intestinal bacteria. Stetson (3, 4) has suggested that the febrile reactions to endotoxin and tuberculo-protein are both manifestations of delayed hypersensitivity and has demonstrated similarities in the systemic and local skin reactions elicited by the two antigens. On the other hand, Hall and Atkins (5) found no correlation between the intensity of delayed dermal sensitivity and the amount of fever elicited by old tuberculin in tuberculin-sensitive rabbits.

The purpose of the present investigation was to learn by passive transfer studies whether the fever produced by the injection of BSA into an immunized animal was a manifestation of delayed or immediate type of hypersensitivity. It was postulated that febrile reactions to BSA occurring in normal recipients given serum from an immunized animal would indicate the transfer of immediate hypersensitivity, whereas similar reactions following the transfer of cells, would suggest the delayed type of hypersensitivity.

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MATERIALS AND METHODS

New Zealand white male rabbits weighing 1.8 to 3.6 kg were employed in all experiments. Temperatures were recorded by placing animals in individual boxes and inserting rectal thermistor thermometer probes to a depth of 10 cm past the anal sphincter, the probes being kept in place throughout the entire experiment. Temperatures were recorded at 5 to 15 minute intervals for 4 to 7 hours, the first hour serving to establish the baseline temperature for a given animal. Animals were conditioned to the temperature procedure by keeping them in the boxes with probes inserted for a total of 10 hours during the 2 days prior to an experiment.

Antigens

Bovine serum albumin (Armour Fraction V) was used as immunizing antigen throughout these experiments. An intravenous challenge dose of 2 mg BSA in 2 ml isotonic saline was used to determine whether an animal was capable of giving a febrile response. When I^{131}-labeled BSA (I*BSA) was used, crystalline BSA (Armour) was labeled by the method of Talmage, Dixon, Bukantz and Dammin (6).

Immunization procedure

Donor animals were immunized with 1-ml subcutaneous injections of a saline in oil emulsion containing 25 mg of BSA in 0.85 per cent saline, Arlecel, and Bayol F in 10:1:9 ratio, respectively. Donors used in serum transfer experiments received one such injection 17 days before bleeding. Donors used in cell transfer studies received two or three monthly injections of BSA in adjuvant, the last injection preceding cell transfer by 1 month.

Methods of transfer

All syringes, bottles, and other instruments were heated at 180° C for 2 hours to render them pyrogen-free.

1) Immune serum pool. Eleven donor animals were exsanguinated under sterile conditions; their blood was collected in separate bottles and the serum separated, pooled, and stored at 4° C in sterile pyrogen-free bottles. Antibody was measured by the ammonium sulfate salting-out technique (7).

2) Normal serum pool. A pool of normal rabbit serum (NRS) was prepared as above.

3) Cells. The method described by Roberts and Dixon (8) was used to transfer approximately 1.2×10^9 splenic

TABLE I

The effect of normal and immune serum transfer upon the febrile response of rabbits challenged with bovine serum albumin

Group	No. of recipients	Serum transfer	Challenge dose	Time of challenge after serum transfer	Fever index
		ml		*hrs*	
		Immune serum			
A	472	45	2 mg BSA	0.5	
	489, 490, 498 502, 503	45	2 mg BSA	2	17.6
	468	60	2 mg BSA	24	
		Normal serum			
B	469, 483, 492, 499, 505, 506	45	2 mg BSA	2	4.0
C	467, 471, 445, 470	0	2 mg BSA	No serum transfer	1.4
		Immune serum			
D	474, 485, 486, 496	45	*a*) 2 ml NaCl	2	2.8
			b) 2 mg BSA	24–48	20.1
		Normal serum			
E	501, 504, 487, 488, 484	45	*a*) 2 ml NaCl	2	2.0
			b) 2 mg BSA	24–48	5.4

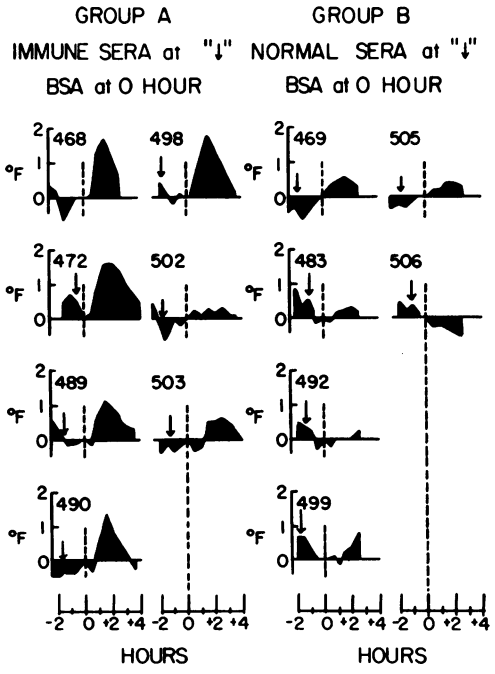

FIG. 1. INDIVIDUAL FEBRILE RESPONSES IN SERUM RECIPIENTS FOLLOWING CHALLENGE WITH BSA. Recipients from group A received 45 ml of primary anti-BSA antiserum, and recipients from group B received 45 ml of normal rabbit serum. Time of serum transfer is indicated by arrow. Groups A and B were both challenged intravenously at time zero with 2 mg BSA (see Table I).

cells (~95 per cent lymphocytes) from immunized donors to normal recipients. Transfer was accomplished within 30 minutes of the time of death of the donor. Spleens from individual donors were teased apart in 7 ml cold polyvinylpyrrolidone-phosphate solution[1] by means of rakes fashioned from 16 gauge nichrome wire gauze. The suspended cells were then injected into several sites in the ventral abdominal wall of the recipient animal.

Fever index

The method of squares was used in calculating the area under fever curves. An arbitrary fever index unit was adopted and is defined as a fever of 0.1° F sustained for 1 hour.

RESULTS

Immune serum transfer (Table I). A 45 to 60 ml aliquot of the immune serum pool was administered intravenously to each of 11 normal recipients. The antibody content of this immune serum as measured by the antigen binding capacity (ABC-33 at 0.2 μg[2]) was 8.7 μg I*BSA N per ml. Simi-

[1] Nineteen parts 3.5 per cent polyvinylpyrrolidone (PVP-Macrose from Schenley Laboratories, Inc.) and 1 part 2 M phosphate buffer, pH 7.2.

[2] The minimal amount of antigen that 1 ml of undiluted serum will bind when 0.2 μg I*BSA is used to test antigen in the ammonium sulfate salting-out technique (7).

lar aliquots of pooled normal serum were also injected into 11 other normal rabbits. One-half, 2, or 24 hours after transfer, seven of the immune serum recipients (group A), six of the normal serum recipients (group B), and four previously untreated rabbits (group C) were challenged with BSA. Two additional groups consisting of four immune serum recipients (group D) and five normal serum recipients (group E) received: *a*) 2 ml of isotonic saline (without BSA) 2 hours after serum transfer and *b*) 2 mg BSA 24 to 48 hours subsequent to the saline challenge.

As illustrated in Figure 1, five of the seven animals that received immune serum followed by BSA (group A) responded with a fever of 1° F or more, whereas no significant fever developed in the animals that received normal serum followed by BSA (group B). Group C (Table I) were normal animals that remained afebrile when given BSA, and represent further evidence that the BSA *per se* was not pyrogenic.

The results obtained in the experiment involving groups A, B and C were confirmed by experiments performed on groups D and E. Group D received immune serum followed by saline and then, within the following 48 hours, was challenged with BSA. It can be seen in Figure 2 that three of the four animals in this group had

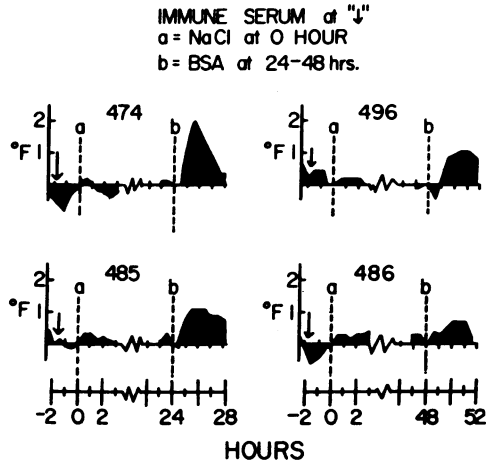

FIG. 2. INDIVIDUAL FEVER RESPONSES TO SALINE AND BSA AFTER PASSIVE TRANSFER OF ANTI-BSA SERUM. Arrow indicates time of transfer of 45 ml of anti-BSA serum to recipients from group D. Vertical line "a" indicates time of saline challenge and vertical line "b" refers to time of BSA challenge (see Table I).

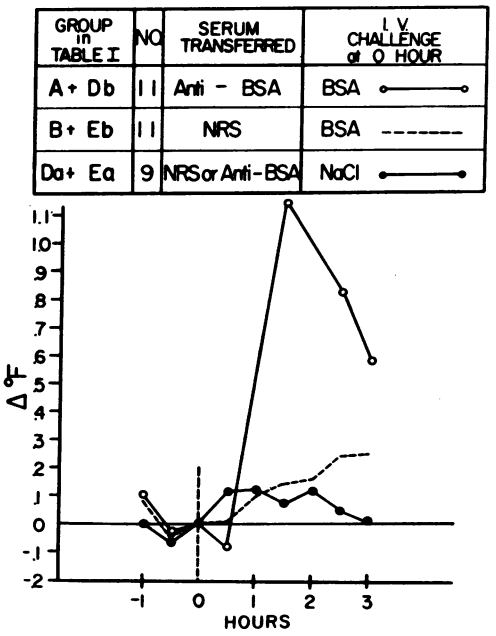

FIG. 3. COMPOSITE GRAPH OF FEBRILE RESPONSES TO BSA IN SERUM RECIPIENTS. The mean febrile response of the 11 animals in groups A and D that received immune serum followed by BSA is shown by the open circles. The mean febrile response of the control groups that received normal and immune serum followed by saline (closed circles) and normal serum followed by BSA (dashed lines) is also shown (see Table I).

a febrile response of at least 1° F when challenged with BSA, while none of the rabbits had a febrile response to the previous injections of immune serum or saline. That the febrile response observed in group D was indeed dependent upon the injection of immune serum is supported by the results obtained in group E. Group E remained afebrile after the injection of BSA although group E was the same as group D, except that normal serum was injected instead of immune serum prior to the challenge with BSA (Table I and Figure 3).

Immune cell transfer. Splenic cell suspensions from immune rabbits were injected into eight normal recipients. The recipients remained afebrile for 2 hours immediately after cell transfer. They also remained afebrile when challenged with 2 mg BSA i.v., 18 hours after transfer. The mean maximal temperature elevation of this group was 0.2° F, and the highest individual temperature elevation was 0.4° F.

DISCUSSION

Fever is a common manifestation of human diseases in which immune phenomena are thought to play a predominant pathogenetic role. However, the role that circulating antibody may play in producing the fever associated with these immunopathologic diseases of man is for the most part still unclear since other fever-producing mechanisms such as delayed type of hypersensitivity, tissue necrosis or inflammation may also be operative in these conditions. An exception to this is the demonstration by Jandl and Tomlinson (9) that the interaction of antibody with erythrocytes can initiate the febrile response.

In the present study, utilizing the rabbit and a BSA-anti-BSA system as an experimental model, it has been possible to produce a state of sensitivity to the pyrogenic action of a soluble antigen by the passive transfer of immune serum. This is considered strong evidence that circulating antibody can play an important role in the initiation of fever. No evidence was obtained to indicate that delayed hypersensitivity played a significant role in this model although it should be noted that the rabbit is a difficult species in which to transfer delayed hypersensitivity passively. The possible importance of delayed hypersensitivity in other species is indicated by the studies of Uhr and Brandriss in the guinea pig (10). These authors were able to produce fever in guinea pigs sensitized with antigen-antibody precipitates [3] at a time when there was a high degree of delayed type of skin reactivity without demonstrable circulating antibody being present.

After challenge with BSA there is a lag period of ½ to ¾ hour before the onset of fever. This lag period resembles that found with endotoxin fever, but is longer. Only a small portion of this time is probably necessary for antigen-antibody complexes to form (11), so that it is unlikely that antigen-antibody interaction per se causes the fever. Rather, it is more probable that antigen-antibody interaction initiates a series of reactions, the end result of which is fever.

[3] Antigens used were egg albumin, tetanus toxoid, human γ-globulin and BSA.

SUMMARY

The relationship of delayed and immediate types of hypersensitivity to the febrile response produced by BSA in previously immunized rabbits was investigated by means of passive transfer studies.

The capacity to respond with fever to BSA was transferred to normal rabbits with large volumes of primary response antisera.

The passive transfer of splenic cells from immune animals to normal animals did not result in the transfer of the capacity to respond with fever upon challenge with BSA.

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