STUDIES ON TUBERCULIN FEVER*

IV. The Passive Transfer of Reactivity with Various Tissues of Sensitized Donor Rabbits

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Fever is a hallmark of tuberculin hypersensitivity. Ever since the classic papers of von Pirquet (1) and the clinico-pathologic observations of Rich (2), fever has been recognized as a cardinal sign of tuberculous infection. The actual events, however, that lead to elevated body temperature in this disease have remained obscure. Many manifestations of tuberculin hypersensitivity, like the skin test, have been attributed to allergy of the delayed type, of which indeed, this disease is the prototype (reviewed in reference 3). On the other hand, tuberculous infection also gives rise to a variety of circulating antibodies whose role in producing any specific manifestations of this form of hypersensitivity is uncertain (4–7).

In regard to fever, a circulating endogenous pyrogen has been noted during experimental fever induced by tuberculin in rabbits sensitized by infection with BCG (bacillus Calmette Guérin) (8, 9). Subsequent in vitro experiments have suggested that blood leukocytes (10), alveolar macrophages (11), and Kupffer cells of the liver (12) may be sources of this material.

By employing the techniques of passive transfer, the work to be reported here has attempted to define the role of various cells as well as of serum factors in mediating tuberculin-induced fever. In addition, the ability of tuberculin to activate certain specific cell types (exudate granulocytes and peritoneal macrophages) in vitro has been investigated.

Materials and Methods

General.—Techniques for processing glassware, needles, and other equipment; preparation and testing solutions to exclude contamination with bacterial pyrogens; housing and selection of rabbits and method of pyrogenic assay have been presented previously (8).

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Sensitization of Donor Rabbits.—The Phipps strain of Calmette-Guérin bacillus was used. Cultures were maintained by alternate passage on Lowenstein slants and Dubos media. Vaccine was prepared from 2-wk old cultures which had been scraped from slants and ground with a mortar and pestle into a fine homogeneous suspension in pyrogen-free saline. Animals were sensitized with an intravenous injection of 2 mg of BCG suspended in 2 ml of saline. This method regularly produces a nodular infection of the lung and a hypersensitive state as evidenced by development of a febrile response of 1–2°C when old tuberculin (OT) is given intravenously (13). These sensitized animals, which were used as donors of exudates and other passively transferred tissues, were injected with 50–100 mg OT, 10–20 days after sensitization, to establish their febrile reactivity. Donors were used several days later to avoid the transient tolerant state that follows immediately after challenge with antigen (8, 13).

Old Tuberculin.—Lot 50 old tuberculin obtained from the Massachusetts Department of Public Health was used as previously (8). This material is stated to contain 2.0 g of OT/ml[1], and was bottled in pyrogen-free glassware. Immediately before use, a small amount was diluted 1:20 in pyrogen-free saline to give a concentration of 100 mg per ml. This dosage was regularly given to test hypersensitivity of all recipients of passively transferred tissues.

Macrophage Exudates.—Macrophages were obtained from peritoneal exudates of hypersensitive animals by the method described by Chase (14). This technique was modified to produce a somewhat higher yield of pure macrophages and to avoid sacrificing the donor animal. Rabbits were immobilized on their backs with extremities extended. The abdomen was shaved and wiped with acetone. No. 15 needles, specially designed with blunted tips and accessory openings along the shaft, were threaded subcutaneously and introduced with gentle pressure into the peritoneal cavity in the midline of the abdomen half way between the xiphoid and the pubis. This method appeared to be virtually painless and only once caused a fatal perforation of the bowel in over 100 collections. 50–100 ml of USP extra heavy mineral oil (pyrogen and bacteria-free) warmed to room temperature was slowly injected intraperitoneally via a sterile 50 ml syringe. Animals were returned to their cages for 4 or 5 days before harvest, at which time they were again immobilized as before and 200–400 ml of pyrogen-free saline were slowly infused intraperitoneally over 15 min with gentle abdominal massage. The immobilized animals with needle still in place were then turned over and secured on a specially designed table with a large oval opening to allow access to the entire abdominal wall. Additional tubing was connected from the intraperitoneal needle to covered flasks, creating a closed system for the exudates to drain by gravity, assisted by massage, over a 15 min period. In some instances, additional pyrogen-free saline (100–200 ml) was flushed into the cavity and allowed to drain. By this means, a homogeneous cloudy exudate was regularly obtained. Prolonged periods of collection over 45 min occasionally produced clotting, even with heparin, and did not seem to enhance cell yield. The total volume of each exudate was slightly less than the volume of saline flushed in at the time of collection (200–600 ml).

An oil-water interface formed in collected exudates immediately at room temperature. The oil was suctioned out and the exudate was immediately contrifuged at 200 rpm for 10 min at 4°C. The supernate was discarded and the packed cells were resuspended in 50 ml of pyrogen-

[1] This figure is equivalent to the international standard for OT. OT 50 has twice the potency of the NIH standard in which the activity has been adjusted so that 0.1 ml of a 1:10,000 dilution contains 0.01 mg OT, as defined by the Commission on Biological Standardization of the Health Organization, League of Nations. (*Quart. Bull.* **4**:515, 1935). This information, as well as the OT used in these studies, was kindly supplied by Leo Levine, Chief of Laboratories, Massachusetts Department of Public Health 375 South Street, Forest Hills, Boston, Mass. 02130. Kjeldahl determinations done in our laboratory indicate that OT 50 actually has about 7 mg protein per ml.

free saline, and recentrifuged at 2000 rpm for 10 min. After a second washing, the cells were resuspended in 50 ml of saline and ready for use. Cells from one or two donors were transferred to a single normal recipient by intraperitoneal injection. After separation of the oil-saline interface, all procedures were carried out at 4°C. Total cell counts were ascertained with a Spencer bright line hemocytometer counting chamber. Differential cell counts were determined from 200 cells on films stained with Wright's stain. Exudates were cultured in beef broth, both before and after the addition of 200 mg streptomycin sulfate and 10,000 international units of penicillin G. After 24 hr incubation, the cultures were streaked on blood agar and in the occasional instances where contamination occurred, results with these exudates were discarded. Individual exudates ranged from 3×10^8 to 2.7×10^9 cells with an average of 1.04×10^9 total cells. Exudates generally contained 85–95% large macrophages with single round nuclei. Some cells showed evidence of phagocytosis of large fat globules, but most cells had clear, homogeneous cytoplasm. Occasional exudates were discarded where the numbers of polymorphonuclear leukocytes exceeded 20%. The interval between collection and utilization of all exudates was less than 5 hr. Cell viability studies were carried out with trypan blue, and on the basis of dye exclusion, viability was regularly over 97%.

Granulocyte Exudates.—Polymorphonuclear leukocytes were obtained by a modification of the above method (15). Rabbits were immobilized as previously described and 350–400 ml of a 100 mg/100 ml solution of shell fish glycogen in pyrogen-free saline was introduced intraperitoneally. 4–6 hr later, the exudates were harvested, washed twice, and resuspended in 50 ml of pyrogen-free saline. The number of cells in each exudate varied from 5×10^8 to 1.65×10^9, with an average of 9.34×10^8. Differential counts showed 77–95% granulocytes with lobulated nuclei and large basophilic intracytoplasmic granules after staining with Wright's stain. Viability studies just before use showed 98 or 99% viable cells.

Sonication of Exudate Cells.—Macrophage and polymorphonuclear leukocyte exudates collected as previously described were subjected to ultrasound at a frequency of 20 kilocycles/sec for 10 min. This method produced a creamy homogenate which showed a finely-divided particulate material and complete dissolution of cellular and nuclear structure on Wright stain smears.

Lymph Nodes and Spleen.—Mesenteric lymph nodes and spleens were removed with sterile precautions as described previously (10). A cellular suspension of each organ was prepared by pressing the tissue through a No. 40 screen and breaking up residual clumps by repeatedly drawing the suspension up and down in a pipette with saline. Cell suspensions were washed and prepared as described above. After removal, tissues were kept at 4°C throughout processing. The average cell yield was 5.25×10^9 cells (with a range of 9×10^8 to 2×10^{10}), virtually 100% macrophages and lymphocytes. The trypan blue method was not effective for determining viability in these preparations, but the time interval between collection and utilization was regularly less than the 5 hr needed for collection and processing of exudates.

Plasma/Serum.—Donor rabbits were lightly anesthetized and heparinized by separate intravenous injections as described previously (10). Blood was obtained by cardiac puncture, centrifuged at 4°C for 30 min at 2000 rpm and the plasma (usually 60–70 ml) removed and stored at 4°C before use. Cultures were obtained as previously described and contaminated material discarded. Serum was obtained and processed in the same manner, except that the donor animal was not given heparin before exsanguination.

Passive Transfer of Cells, Plasma, and Serum.—In earlier experiments, cell suspensions obtained from donor rabbits were injected intraperitoneally with specially prepared needles (see above) into recipient rabbits. Most animals, including all recipients that had been used in earlier transfer experiments, were demonstrated to be nonresponsive to OT several days before use. A few freshly acquired rabbits were used as recipients without prior testing. However, it was found later that some "normal" animals had spontaneously developed a febrile reactivity to tuberculin after prolonged residence in the colony, presumably due to inap-

parent cross-infection, as has been reported with febrile responses to staphylococcal antigen (16). Thereafter, all recipients, regardless of time of acquisition, were routinely tested with OT before use.

In some experiments, saline suspensions of various tissues from sensitized donor rabbits were given intravenously, as were all passive transfers of serum and plasma.

RESULTS

Passive Transfer of Peritoneal Macrophages.—

Peritoneal cells (80–98% mononuclear cells) were harvested from exudates induced by mineral oil in 12 BCG-sensitized donor rabbits (see Methods). Each exudate was injected intraperitoneally into a single normal recipient. The individual dose of cells varied from 3.0×10^8 to 2.7×10^9 with an average of 1.04×10^9. The recipient rabbits were then tested with OT 48 hr after receiving passively transferred cells. Thereafter, because of the pyrogenic tolerance that regularly develops to daily injections of OT, each animal was tested at 2 or 3 day intervals for a total of three or more challenges, as long as a positive febrile response (0.3°C) was elicited. Of the 12 animals in the group, 10 had one or more febrile responses to OT during the 2–3 wk interval after passive sensitization with cells. Fig. 1 A depicts the febrile responses to OT observed in a single recipient.

In general, these responses were typical of the group, although most animals had fever when first tested on day 2. Fever developed after a latency of 30–45 min with a sharp peak at 1 ½–1 ½ hr and gradual defervescence over a 3 hr period. Although monophasic rather than biphasic, the reactions resembled those produced by tuberculin in the BCG-infected donor animals (see Fig. 1). The composite responses of the 10 positive reactors is expressed as average maximal height of fever, and shown in Fig. 2 A. Responses were positive from 48 hr to the 14th day after transfer, and then gradually subsided. Most animals tested later than 2 wk after trasfer were unresponsive to OT.

Passive Transfer of Lymph Node and Spleen Cells.—

To determine if monocytes derived from spleen and lymph nodes of BCG-sensitized donor rabbits might similarly transfer this form of reactivity to OT, preparations of these tissues were obtained and passively transferred in the same manner to normal recipients (see Methods). Individual cell doses varied from 9×10^8 to 2×10^{10}, with an average of 5.25×10^9.

All 10 animals tested serially with OT developed febrile responses that generally paralleled the results seen with peritoneal monocytes (see, for example, Fig. 1, B and C). The average responses, shown in Fig. 2 B, became maximal 7 to 14 days after transfer and gradually declined thereafter, a pattern that resembled the reactions in animals given peritoneal monocytes, except that fewer animals given spleen or lymph nodes developed febrile responses to OT 48 hr after transfer.

Passive Transfer of Sonicated Preparations of Peritoneal Monocytes.—The above experiments suggested that mononuclear cells from these three sources were capable of passively sensitizing recipients to antigenic challenge with

tuberculin. Since these transferred cells were viable, the following experiment was devised to determine if similar results would be obtained with factors derived from sonicated cells.

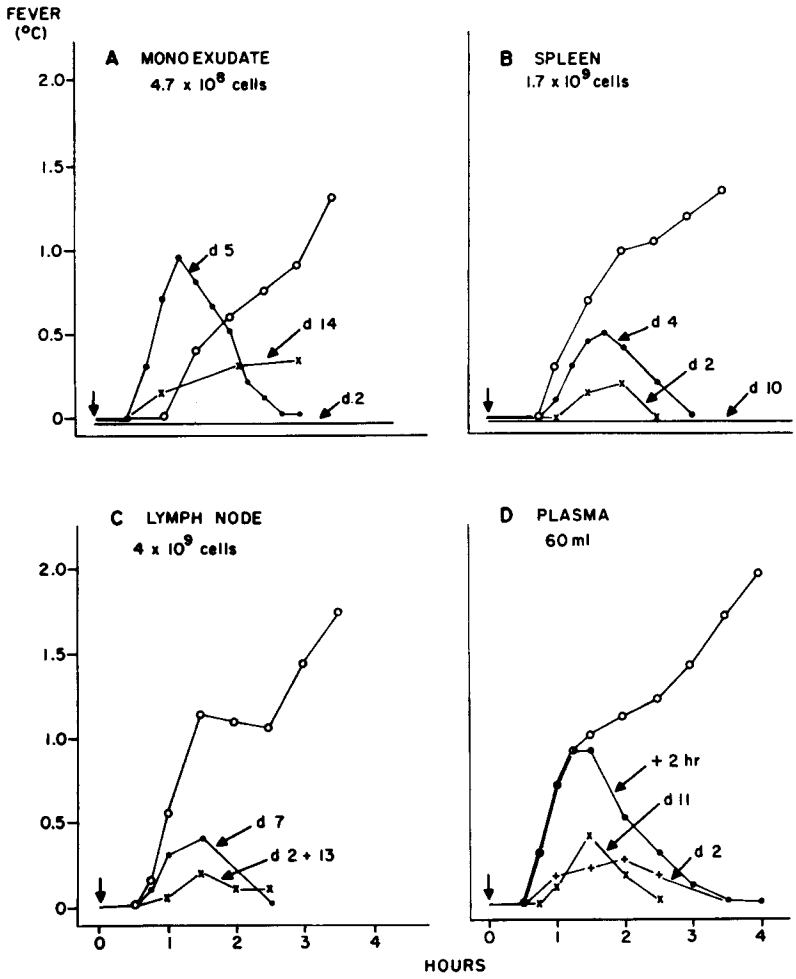

FIG. 1. Typical febrile responses (closed circles) of normal recipients to tuberculin given at various intervals after passive transfer of cells or plasma from BCG-sensitized donor rabbits. Open circles indicate responses of the donors to tuberculin. d, day after transfer.

Peritoneal monocytic exudates were obtained by the same techniques as previously and subjected to ultrasound in order to disrupt cell membranes (see Methods). The preparation was then given intraperitoneally to recipients and their reactivity to tuberculin serially tested as in the previous experiments.

No significant degree of hypersensitivity, as evident by the development of a febrile response to OT over a period of 2–7 days, was obtained in six experiments.

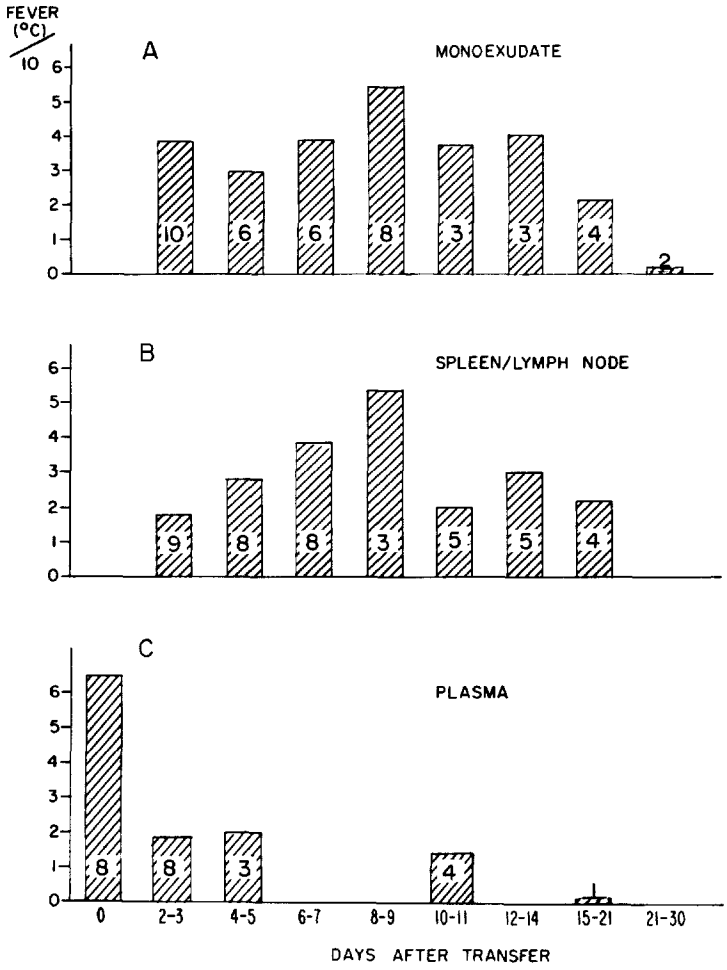

FIG. 2. Average fevers of normal recipient rabbits given 100 mg OT at intervals after passive transfer of various tissues from BCG-sensitized donors. Numbers within bar graphs ndicate number of recipients.

Passive Transfer of Polymorphonuclear Leukocytes from Sterile Exudates.—To determine whether granulocytes might also passively transfer the capacity to react to tuberculin with fever, a similar series of experiments were performed with acute granulocytic exudates obtained from sensitized rabbits (see Meth-

ods). Individual cell doses ranged from 5×10^8 to 1.65×10^9 with 97 to 98% polymorphonuclear leukocytes, almost all of which were viable by dye exclusion. No significant febrile response to tuberculin could be elicited when normal recipient animals were serially challenged 2–13 days after passive transfer in eight experiments.

Plasma and Serum Transfer.—The preceding experiments suggested that the passive transfer of this form of hypersensitivity fever could be accomplished with immunologically competent, viable cells.

To determine whether this phenomenon depended upon cells uniquely, like other manifestations of delayed hypersensitivity, or whether humoral factors might also be involved, a group of eight recipient animals were each intravenously injected with 30–60 ml of plasma from hypersensitive animals. 1–2 hr later, the recipients were tested for their capacity to develop fever to tuberculin. Fig. 1 D shows a representative response.

Typical febrile responses were elicited in six of eight animals. When tested again 2 days later, however, most of these animals failed to develop a significant response, possibly due to the removal of antibody in immune complexes. Similarly, later injections of tuberculin, from days 5 to 15 after transfer, usually failed to evoke fever at a time when responses to the passively transferred mononuclear cells were maximal, as shown previously. (Compare Figs. 2 A and 2 C).

Since the preceding experiment involved transfer of plasma to some recipients which had been used in earlier passive transfer experiments with cells and which had subsequently become negative to tuberculin, the findings were confirmed with 10 additional, normal rabbits given either plasma or serum (5 recipients each) and tested with OT at various intervals thereafter to determine the duration of such passively transferred sensitivity.

Positive transfers were obtained in 7 of the 10 recipients with an average of 0.8°C in those tested within 24 hr. Two recipients that were initially tested 24 hr after passive transfer of serum or plasma had vigorous febrile responses to tuberculin. No response was obtained in a single recipient that was initially tested 4 days after transfer of plasma, although the other recipient of plasma from the same donor had a markedly positive reaction to OT given on the same day as the plasma. Plasma and serum appeared to be equally able to transfer this form of reactivity and, as seen previously, recipients in these confirmatory experiments had lost most of their reactivity when subsequently tested with OT on the 8th day or later after transfer.

In seven experiments, involving passive transfer of similar quantities of normal serum or plasma to normal recipients, a febrile response to tuberculin given several hours later was only obtained in one instance (0.6°C).

Activation of Exudate Cells by Tuberculin In Vitro.—Since evidence from previous work utilizing blood leukocytes suggested that the granulocyte was

activatable by OT in vitro (10, 11), it was decided to test this inference more directly by adding OT to polymorphonuclear cells derived from acute peritoneal exudates.

Cells from glycogen-induced exudates were harvested at 4–18 hr from BCG-infected donor rabbits (see Methods). Differential cell counts established that the per cent granulocytes in such preparations varied from 40 to 99%. After being washed twice in saline, the cells were resuspended in about 25 ml normal serum or plasma in order to suppress further release of EP from these already activated cells (15). The cell-serum suspension was then divided in half and tuberculin was added to one of the two fractions in a dosage of 100 mg per 1–2 $\times$

TABLE I

*Mean Fevers Produced by Supernates of Peritoneal Exudate Cells (Derived from either BCG-Sensitized or Normal Donors) and Incubated for 5 Hr with or without OT In Vitro**

Donor		Exudate			Recipient		
Sensitized	Number	Type	Cells ($\times 10^7$)		Number	Average fever	
			PMN	M		+ OT	Control
						°C	°C
A	2	Glycogen	7–19	0.8–1.4	10	0.72	0.29
B	3	Glycogen	5–14	3–4	8	0.64	0.22
C	4	Glycogen	5–16	5–11	16	0.65	0.17
D	3	Mineral oil	0.8–2.3	6–9	8	0.33	0.07
E	6	Mineral oil	0.75–3.5	5–24	9	0.56	0.15
Unsensitized	6	Glycogen	9–28	<1–5	28	0.39	0.27

* Numbers of granulocytes (PMN) and mononuclear Cells (M) indicate range of cells from which each dose was derived. See text for details.

10^8 cells. Both the experimental and control (cells alone) samples were then incubated for 5 hr at 37°C. At the end of this period, the cells were centrifuged (2000 rpm for 30 min) and the supernates injected into normal rabbits in a dose equivalent to 1–2 $\times$ 10^8 cells to assay the amount of EP produced. To minimize the variability in responsiveness of different rabbits, each recipient received both experimental and control samples, usually on the same day and in random order.

The results are shown in Table I. In this table, the glycogen-induced exudates (A–C) are grouped according to the number of mononuclear cells present. The number of granulocytes, on the other hand, was relatively constant (average of 11 $\times$ 10^7).

It is apparent that the average fever induced by these three groups was approximately the same, despite an increase in the number of monocytes from an average of 1 $\times$ 10^7 to 8 $\times$ 10^7 (cf. groups A and C). Conversely, in group D, comprising chronic exudates induced by mineral oil, in which the granulocyte count was markedly reduced (average 1.5 $\times$ 10^7) while the number of monocytes (average 7.5 $\times$ 10^7) was nearly equal to that of the granulocytes in the preceding groups, the average fever was only half that produced by groups A–C.

These data, therefore, suggest that the granulocyte is chiefly responsible for the EP evolved under these conditions.

If present in sufficient numbers, however, mononuclear cells from peritoneal exudates may also release significant amounts of EP after a short period of incubation with OT in vitro, even under these unfavorable conditions (17), as shown in group E in Table I. Here the average number of mononuclear cells per dose (14.5×10^7) was about twice that of group D (7.5×10^7) and the degree of fever induced was nearly twice as great.

These data appear to confirm work of Hahn et al. (17) that peritoneal monocytes, like monocytic cells derived from other tissues (11, 18) are a source of EP and can be activated by appropriate stimuli.

Granulocytic exudates from unsensitized donors, on the other hand, were little, if at all, activated to produce EP in vitro when incubated with OT under the same conditions (cf. group A with "unsensitized" group). This reaction, therefore, appears to require specifically sensitized cells and is not due to a nonspecific "toxic" action of OT on exudate cells (19, 20).

Passive Transfer of Granulocytes to Recipients Given OT on the Same Day.— The preceding experiments have demonstrated that tuberculin can activate granulocytes in vitro and that serum or plasma from sensitized donors passively transfers pyrogenic reactivity to tuberculin in normal recipients, perhaps, as suggested in earlier work (10), by sensitizing their circulating granulocytes. Hence, it seemed appropriate to determine if passive transfer of exudate granulocytes from BCG-infected donors to normal recipients would permit these animals to react to tuberculin given several hours later. Although granulocyte preparations given intraperitoneally were unable to sensitize normal recipients to react with a febrile response to OT when the OT was initially given 48 hr later, it was reasoned that such cells, when given intravenously and still viable, might be able to react with OT in the bloodstream and release EP within the host.

Accordingly, a group of five recipients were each injected intravenously with approximately 1×10^9 cells (containing >95% granulocytes) freshly harvested from glycogen-induced exudates in BCG-infected donor rabbits. The cells were washed once and resuspended in saline before injection. About 2 hr later, after the initial, transient febrile responses to the EP contained in such exudates had subsided and the temperatures had stabilized, each recipient was given 100 mg OT.

Four of the 5 recipients responded with typical tuberculin fevers (0.75 to 1.4°C) after latent periods of 45–60 min. The other recipient developed an equivocal fever (0.3°C) after a latent period of 2 hr. The average response of all five recipients is shown in Fig. 3. When these recipients were tested 6 and 9 days later, the average response of the four reactive animals had fallen progressively to less than 0.4°C.

The data suggest, therefore, that granulocytes of sensitized donor rabbits

may react directly with OT in vivo to release EP, just as they appear to do in vitro.

Passive Transfer of Other Cells to Normal Recipients Given Tuberculin on the Same Day.—Since tuberculin fever was induced in normal rabbits shortly after they had been transfused with exudate granulocytes from sensitized donors, analogous experiments were performed with other tissues of BCG-infected donors. If the fever induced by OT were derived from EP released by the transferred cells, it was reasoned that this response would appear only in recipients receiving cells that were capable of releasing EP when incubated with tuberculin in vitro.

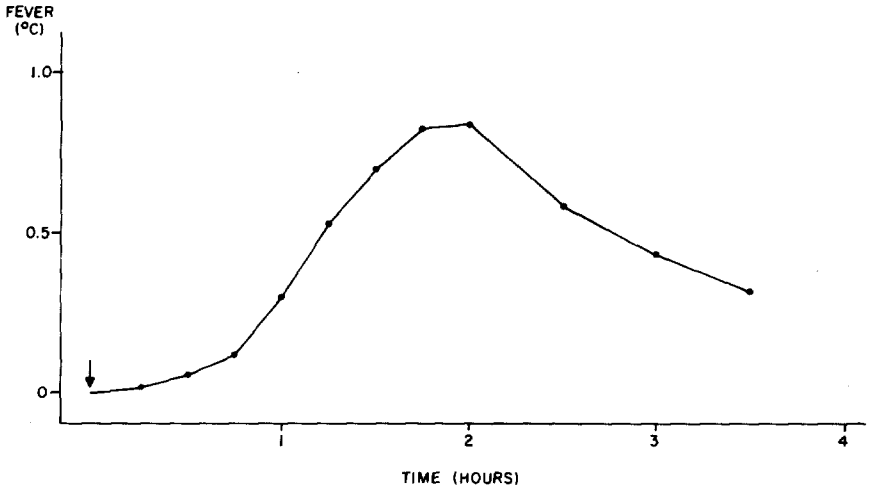

FIG. 3. Average febrile response of five normal recipients to 100 mg tuberculin given 2 hr after intravenous injection of approximately 1×10^9 exudate granulocytes from BCG-sensitized donor rabbits.

Accordingly, new recipients, almost all of which had been previously tested several days before with OT to insure a negative response, were intravenously injected with saline suspensions of the following tissues derived from BCG-sensitized donor rabbits: (*a*) lung alveolar cells, (*b*) spleen (*c*) mesenteric lymph nodes, and (*d*) blood cells. Individual dosages varied from 3×10^8 to 1.3×10^9 leukocytes. Techniques for obtaining and processing these cells have been presented previously (11). To obviate occasional instances of sudden death after these suspensions, most recipients were injected with 1000 units of heparin. Several hours later, if the temperature had remained stable after the tissue suspension had been injected, the recipients were challenged with 100 mg OT.

The average results both for OT given on the same day (d O) and again on d 4–6 are shown in Table II. A majority of recipients in all groups developed febrile responses to tuberculin when given on the same day, although from pre-

vious work we have found that of these tissues only the lung and blood cells release EP when incubated with OT in vitro (11). These results, then, suggest that OT does not mobilize EP from the transferred spleen and lymph node donor cells, but perhaps causes these cells to release an intermediate factor capable of rapidly activating the recipient's cells to produce EP. The nature of this factor has not been established, but since an analogous experiment in which normal tissues were transfused into normal recipients failed to establish a pyrogenic reactivity to OT, it seems likely that some form of immunological reaction between tuberculin and the specifically sensitized donor cells is the initial event leading to the release of EP and fever in the recipient of the passively transferred cells.

TABLE II

*Mean Fevers Produced by 100 mg Tuberculin in Normal Recipients After the Intravenous Inoculation of Saline Suspensions of Leukocytes Derived from Four Tissues of BCG-Sensitized Donor Rabbits**

Lung		Spleen		Lymph Node		Blood	
d 0	d 4–6	d 0	d 4–6	d 0	d 4–6	d 0	d 4–6
0.82 (15)	0.34 (14)	0.94 (14)	0.81 (9)	0.63 (10)	0.56 (10)	0.91 (10)	0.34 (9)

* Figures represent responses to tuberculin given on the same day (d 0) and again 4–6 days later. Numbers in parentheses indicate number of recipients. See text for details.

Of particular interest are the results obtained when these recipients were rechallenged with the same dosage of OT 4–6 days later (see Table II). In the groups receiving lung and blood preparations—tissues containing largely cells that are immunologically incompetent, but capable of reacting with OT in vitro to release EP—the second response to OT was almost invariably less than the first (20/22 instances). With the groups receiving immunologically competent tissues, spleen and lymph node, however, the second response was often higher than the first (10/19 instances) with average responses in both instances approaching the initial fever suggesting, as in the earlier experiments reported here, that the recipients had been sensitized by these cells to react subsequently to OT.

DISCUSSION

Following the initial demonstration by Chase that delayed hypersensitivity could be passively transferred with cells (14), this phenomenon has been repeatedly confirmed and its mechanism intensively investigated (reviewed in reference 21).

Generally, guinea pigs have been used in transfers of this type with the usual determination being the characteristic delayed skin reaction to the specific antigen. In other instances, fever, shock, and death have been studied as systemic manifestations of delayed hypersensitivity. In previous work, we have shown that the febrile response of hypersensitive rabbits to intravenously injected OT is a sensitive and reproducible manifestation of tuberculin hypersensitivity (8, 10, 13). Whether delayed or immediate form of allergy or both are responsible for tuberculin-induced fever has not, however, been established. Tuberculin fever is associated with the appearance of a circulating endogenous pyrogen (EP) which is a common mediator of many experimental and, by implication, clinical fevers induced by various microbial and nonmicrobial agents (22). In studies carried out in vitro, both blood leukocytes and lung macrophages (11) and, more recently, the Kupffer cells of the liver (12) have been shown to be potential sources of EP in this form of hypersensitivity fever. Despite earlier reports (23, 24) spleen and lymph node cells do not appear to be activatable by tuberculin in vitro (10, 11, 25), although they may release EP with certain other stimuli (11, 12).

Normal blood cells incubated for short periods with tuberculin in the plasma of sensitive rabbits have been found to release EP in vitro (10). This finding suggests that humoral factors are also involved. The present work was designed to determine, by supplementary studies in vivo, what role these cellular and humoral factors play in the development of tuberculin-induced fever.

The passive transfer experiments with peritoneal monocytes, lymph node and spleen cells indicate that hypersensitivity, as measured by fever, reached its maximum during the second week after transfer and gradually declined thereafter (see Fig. 2). Since neither spleen nor lymph node cells appear capable of reacting with OT in vitro to release EP, it seems unlikely that OT causes fever in the recipient by activating the passively transferred cells. However, the donor cells may sensitize other EP-producing cells in the recipient by direct cell to cell contact, or by releasing antibody. Alternatively, after contact with antigen in the new host, the sensitized donor lymphocytes may release a factor which nonspecifically activates appropriate cells of the recipient to produce EP. Like the production of various cytotoxic (26) or macrophage-inhibiting factors (27–29), such a mechanism may not require circulating antibody. Although the results presented here do not allow a clear choice between these alternatives, the positive transfers of reactivity after 48 hr suggest that immunologically competent donor cells are producing a biologically active humoral substance, possibly antibody, in the recipient. This substance may then be adsorbed onto polymorphonuclear leukocytes or other activatable cells (30–32) which are stimulated to release endogenous pyrogen by antigenic challenge with tuberculin (10). Several lines of evidence seem to support this hypothesis. Only immunologically competent cells were able to passively confer hypersensitivity evident after 48 hr. Positive transfers after this interval were not elicited by polymorphoncuclear leukocytes, or by nonviable cells.[2] The observation that

[2] These results also suggest that recipients were not actively sensitized by antigen (contained in OT) carried over with the transferred cells.

sensitivity as measured by fever was most marked during the 2nd wk after passive transfer and declined rapidly thereafter suggests that the transferred cells were functioning as a "protected clone" and that during this time they were producing antibody until the cells were later rejected by the host. Since sensitivity was transient, it seems improbable that the donor cells had stimulated the host's own cells to become immunologically active. Further, since passive sensitization was achieved only with viable cells, we were unable to implicate a biologically active substance such as "transfer factor" (33–38) in sensitizing the recipient's cells to react with antigen and produce EP after an intravenous injection of tuberculin. The possibility that sonication might destroy a transfer factor associated with the sensitized donor cells cannot be excluded, but seems unlikely (33). The clearest evidence that a humoral factor can mediate tuberculin fever was the successful transfer of reactivity with either serum or plasma (see Fig. 1 D). This finding would seem to confirm earlier in vitro evidence (10) that a circulating substance, presumably antibody sensitizes polymorphonuclear leukocytes, and perhaps cells of the monocytic series, (via cytophilic antibody) so that these cells release EP when subsequently exposed to antigen.

On the other hand, the results demonstrating that animals given an intravenous inoculation of spleen or lymph nodes from sensitized donors can respond with fever to tuberculin given 2 hr later, suggests that sensitized cells may transfer factors other than humoral antibody that play a role in producing the EP that mediates this response. With exudate granulocytes, as well as blood leukocytes and lung macrophages, it seems probable that some of the EP may be derived from the passively transferred cells, since these cells can generate EP in vitro after incubation with OT. However, passively transferred spleen and lymph node cells, which do not appear to be activatable by OT in vitro, presumably respond to the intravenous inoculation of OT by liberating either a cell-bound antibody or an intermediate substance that either nonspecifically. or in conjunction with antigen, activates the recipient's own cells to produce EP.

Although these data suggest that such a substance is released or produced in vivo, we have been unable so far to demonstrate its existence in vitro. When tuberculin is incubated with lymph nodes of sensitized donor rabbits, the supernates of such mixtures do not appear to contain substances that will sensitize normal blood leukocytes (10) or alveolar macrophages (unpublished data) to react with OT and release EP after brief periods of incubation in vitro.

SUMMARY

Utilizing techniques of passive transfer, we have investigated the factors responsible for production of fever when tuberculin is given intravenously to specifically sensitized rabbits.

The ability to develop a febrile response to tuberculin could be passively transferred to normal recipients with viable mononuclear cells from peritoneal

exudates, spleen, or lymph nodes of donor rabbits sensitized with BCG. Sensitivity was usually apparent 48 hr after transfer, maximal at 7 to 14 days, and rapidly declined thereafter. Granulocytes and nonviable, sonicated, mononuclear cells from similarly sensitized donors were unable to transfer this form of reactivity.

Passive transfer of reactivity was also effected with plasma and serum, suggesting that the reaction of antibody with antigen contained in tuberculin is one of the initial steps by which the host cells are activated to release the endogenous pyrogen (EP) that mediates this form of hypersensitivity fever.

An intravenous infusion of granulocytes, as well as of several types of mononuclear cells from sensitized donors, made most recipients responsive to the pyrogenic effect of old tuberculin (OT) given 2 hr later. Some of these passively transferred cells, such as the granulocyte and alveolar macrophage, may be activated in vivo by OT, as they are in vitro. However, in the case of splenic and lymph node cells that cannot be activated by OT to produce EP in vitro, it seems likely that an intravenous injection of OT causes these transferred, sensitized cells to liberate an intermediate substance that either directly, or in association with antigen, activates the host's normal cells to produce EP.

In support of previous suggestions that leukocytes of several types, as well as phagocytic cells of the reticuloendothelial system, serve as potential sources of EP in tuberculin-induced fever, evidence was presented that OT also activates both granulocytes and mononuclear cells from sterile exudates of BCG-sensitized donors to produce EP in vitro.

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