Adjuvant arthritis in the rat*

Effect of intraperitoneal injections of either whole dead mycobacteria or tuberculin

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The total immune response made by an organism on encountering an antigen is conditioned by previous exposure to that antigen and, to a lesser extent, by exposure to other antigens. Conventional immune reactions may thus be augmented (in the secondary response), reduced (by immune tolerance or immune competition), or selectively modified (by immune deviation).

The experimental arthritis of adjuvant disease in the rat is probably immunologically determined, being a cell-mediated response to the same antigenic component of the tubercle bacillus (TBC) which endows this organism with its conventional adjuvant properties. The predominantly articular distribution of the lesions is presumably due to local joint conditions in some way favouring the settling out there of minute fragments of mycobacteria absorbed from the injection site.

As with other immune reactions, adjuvant arthritis can be modified by previous exposure to the responsible antigen. It is prevented by the administration of TBC either in the neonatal period (Houssay and Frangione, 1961), or during the period immediately before the sensitizing injection (Gery and Waksman, 1967); and it can be reduced by immune competition (Pearson and Wood, 1962).

Most interest in the modification of immune responses by the administration of antigen has centred on experiments in which the modifying dose is given before the definitive antigenic challenge. However, consideration of the possibility that this type of immune manipulation may offer an approach to the treatment of immunologically determined disease raises the question of the extent to which it is possible to modify reactions already under way. This principle is already applied in the treatment of atopic disorders by 'desensitizing' injections, which stimulate the production of blocking antibody. The question therefore arises whether other types of immuno-pathological processes may be inhibited by using antigenic stimulation to modify the immune status of the subject.

To explore this possibility in an experimental disease model, an attempt has been made to modify the course of adjuvant arthritis in the rat by injection of either whole TBC or tuberculin (protein purified derivative—PPD) given during the incubation period of the disease. In the event, whole TBC protected the animals from the disease, while PPD tended to aggravate the lesions.

Methods and materials

Animals
Colony-bred strains of Sprague-Dawley, Hooded, and Wistar rats of either sex, but weighing over 150 g. were employed. In any one experiment, all groups consisted of the same strain and sex, and groups were approximately matched for weight.

Induction of arthritis
Each animal received an intracutaneous injection into the right hind foot-pad, consisting of 0.6 mg. dried heat-killed TBC (Central Veterinary Laboratory, Weybridge) homogenized in 0.1 ml. heavy mineral oil (liquid paraffin, B.P.). The injection was made on 'Day 0'.

Quantitation of arthritis
The method used for scoring joints has been described (Currey and Ziff, 1968). The score for the injected foot was excluded from the total.

Intraperitoneal injections
Unless otherwise stated, these consisted of the antigen, or other substance, made up to 1 ml. in physiological saline and injected intraperitoneally on Day + 5. Control animals received 1 ml. saline alone. Substances injected consisted of whole heat-killed, dried TBC ground and homogenized in the saline; PPD (Central Veterinary Laboratory, Weybridge); washed sheep red blood cells

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adjuvant (FCA) rats after indicated in the one received FIG. (obtained in Alsever’s solution from Burroughs Wellcome and Co.); pertussis vaccine, initially $40 \times 10^6$ organisms per ml. (Burroughs Wellcome and Co.); and aluminium hydroxide gel ('Alhydrogel', Superfos, Copenhagen).

Skin testing
The same preparations of TBC and PPD used for intraperitoneal injections were diluted to 1 mg./ml. and 10,000 u. (0.2 mg.)/ml. respectively and, using a very fine (30 gauge) needle, 0.02 ml. of these dilutions were each injected into the pinna of one ear of animals to be tested. A micrometer was employed to measure the thickness of the ear at the injection site immediately before the injection, and 24 and 48 hours later.

Results
Fig. 1 shows the effect of giving either whole TBC (5 mg.) or PPD (200 u. = 0.004 mg.) by intraperitoneal injection on Day +5. Compared with the control group of animals, those receiving whole TBC showed a marked reduction in arthritis. By contrast, the PPD group developed slightly more marked lesions. Fig. 1 also shows the progressive mean weights of the rats in each group. Two influences are apparent here: the intraperitoneal injection of whole TBC produced a temporary weight loss lasting 2 days which did not occur in the other two groups. Then, once the clinical lesions appeared, the TBC group gained weight steadily, compared to marked loss of weight in the control group, and an even greater loss in the PPD-treated animals. These weight changes correlated with the general appearance of the rats, the TBC group being more vigorous, and clearly having been protected, in a general sense, from the disease.

A series of further experiments showed these results to be reproducible. Although the severity of the adjuvant arthritis varied considerably from experiment to experiment (depending on the strain of rat, the batch of adjuvant, and apparently other, undefined variables), nevertheless the trends observed in this first experiment were consistent.

With the method of scoring employed, the high joint scores recorded in control animals meant that there was little scope for the arthritis-aggravating influence of PPD to be apparent. However, when a less sensitive strain of rat was used, the PPD-treated animals developed an obviously more severe arthritis. Such an experiment is illustrated in Fig. 2.

![Fig. 2](https://example.com/fig2.png) Fig. 2. Result of an experiment similar to that illustrated in Fig. 1, but employing a strain of Wistar rat less susceptible to adjuvant arthritis. Lower joint scores in the control (saline-injected) animals allow the enhancing effect of the PPD injection to be apparent. A larger dose of PPD (0.1 mg. = 5,000 u.) was employed. Each group consisted of six rats.

Variation in the dosage
The results of the initial experiment raised the question whether the opposite effects of TBC and PPD might be a function of dosage. However, it is difficult to know what should be regarded as equivalent doses of these two compounds in the context of this experimental model. Mycobacteria are chemically very heterogeneous and contain what is probably the responsible antigen in small quantities, while PPD is a more concentrated protein extract which apparently does not contain this particular antigen. A series of further experiments were therefore carried out in an effort to establish the dosage.
range over which these effects were apparent. The results are summarized in the Table. TBC produced inhibition in doses down to as small as 0.1 mg. Below that the effect was variable, but showed no evidence of significant aggravation. PPD aggravated the arthritis in doses up to as large as 5,000 u. (0.1 mg.). Higher doses produced variable results, tending generally to be inhibitory.

### Table Adjuvant arthritis: Influence of intraperitoneal injections given on Day +5

<table>
<thead>
<tr>
<th>TBC (mg.)</th>
<th>PPD (mg.: units in parenthesis)</th>
<th>Exacerbation</th>
<th>Inhibition</th>
<th>Exacerbation</th>
<th>Inhibition</th>
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</table>

### Variation in timing

Some degree of inhibition of arthritis was achieved with TBC administered as early as Day +2 and as late as Day +11. These results were not sufficiently clear-cut to indicate the optimal day for injection, but Day +5 appeared to be as effective as any, and was used for most of the experiments. Some animals injected on Day +2 developed a milder delayed arthritis starting on Day +17 and reaching a maximum on Day +20. This ‘escape’ was not apparent in the other groups.

### Variation in the route of injection

Experiments in which the intraperitoneal injections were replaced by the same substances given by intramuscular injection, gave variable results which were difficult to interpret. In general it appeared that intramuscular injections were a less effective method of producing a qualitatively similar effect.

### Injection of other substances

The effect was studied of giving intraperitoneal injections of other substances on Day +5. These included a range of doses of pertussis vaccine, aluminium hydroxide gel, and washed sheep red blood cell suspensions given both as a primary and as a secondary challenge. These injections introduced considerably more variation in the severity of the subsequent arthritis, and all three substances, in individual experiments, appeared to produce some aggravation of the arthritis. However, no consistent trend was apparent comparable to that seen with TBC or PPD.

### Skin testing with TBC and PPD

Rats injected with Freund’s complete adjuvant in the manner necessary to produce arthritis, also develop delayed-type skin responses to both PPD and whole TBC in suspension. This is conveniently quantitated by the ear injection technique described above under Methods. Fig. 3 shows the results of an experiment similar in design to that illustrated in Fig. 1, but in which all animals were skin tested on Day +12. It is clear that the groups injected with adjuvant developed positive skin tests to both PPD and TBC, in contrast to the normal rats included as controls. There also appears to be some tendency for the intraperitoneal TBC injections to decrease both skin responses, while the PPD injections make the skin responses greater. Such an action would be very interesting in view of the similar influence of these injections on the arthritis. However, analysis shows that these apparent differences did not reach statistical significance. In individual animals within the groups, there did not appear to be any correlation between the joint score and the intensity of either type of skin response. Rats given either type of intraperitoneal injection alone, and not receiving an adjuvant injection, gave negative skin tests 7 days later.

![Fig. 3 Delayed responses to skin tests using PPD and TBC. Animals given Freund’s complete adjuvant (FCA) developed positive tests compared with untreated control rats (C). The intraperitoneal injection on Day +5 of 5 mg. whole dead mycobacteria (TBC) or 200 u. tuberculin (PPD) appeared to modify slightly the intensity of this positive response compared with that seen in animals receiving intraperitoneal saline (NS).](http://ard.bmj.com/)

### Peritoneal histology

When rats were killed on Day +21, those that had received intraperitoneal injections of TBC showed increased vascularity and adhesion formation in the peritoneum. Sometimes there was an excess of fluid and macroscopic granuloma. Microscopic examination confirmed the presence of granuloma (Fig. 4). Peritoneal histology in animals receiving PPD ranged from normal to mild non-specific inflammatory changes (Fig. 5) which were sometimes present also in saline-injected animals.
Adjuvant arthritis in the rat

FIG. 4 Photomicrograph, showing granulomata in posterior peritoneum of a rat. Specimen obtained on Day +21 from an animal which had been protected from developing adjuvant arthritis by an intraperitoneal injection of whole dead tubercle bacilli (1 mg.) given on Day +5. Haematoxylin and eosin × 80.

FIG. 5 Photomicrograph, showing non-specific inflammatory changes in posterior peritoneum of a rat. Specimen obtained on Day +21 from an animal in which adjuvant arthritis had been aggravated by an intraperitoneal injection of 0.1 mg. PPD on Day +5. Haematoxylin and eosin × 80.
Discussion

These results show that an intraperitoneal injection of dead TBC during the incubation period is a highly effective method of inhibiting adjuvant arthritis in the rat. This effect is specific to the extent that it is not produced by other adjuvants (pertussis vaccine and aluminium hydroxide gel) nor by another strong antigen (sheep erythrocytes). By contrast, PPD injections, over a wide dosage range, produced actual enhancement of the arthritis.

These experiments are too crude to allow a precise interpretation of how the mycobacterial injections are acting. The fact that the injected animals are healthier in a general sense, judged by weight gain and general appearance, as well as manifesting less arthritis, suggests that this is a true protection from the disease.

The production of adjuvant arthritis in the rat requires very precise conditions to be satisfied, indicating perhaps that it depends on a critical state of immune reactivity. If so, the intraperitoneal TBC injection might operate either by immediately altering this immune state (e.g. by shifting the balance between cell and antibody-mediated responses) or by providing an alternative, larger, and more accessible focus of antigen to which the specifically instructed cells mediating delayed-type immune responses might be preferentially attracted. The peritoneal cavity is well suited to such a role, providing a large surface area across which inflammatory cells pass easily, and in which these cells can, on meeting the antigen, mount a granulomatous reaction without producing any critical functional derangement in the animal as a whole. This scheme ascribes to the peritoneal cavity the role of a sump, in which immunologically competent cells are trapped, leaving insufficient cells to mount a reaction against the small fragments of TBC disseminated from the original injection site. It implies too that the actual number of sensitized cells available to react against a particular antigen may impose a finite limit on the extent of the inflammatory response.

Adjuvant arthritis is normally a self-limiting disease, and it is noteworthy that the TBC injections (unlike corticosteroids—Newbould, 1963) prevent rather than postpone the disease. The aggravation of adjuvant arthritis by PPD is an interesting, but unexplained observation. It is somewhat reminiscent of the exacerbation of human pulmonary tuberculosis which may follow an injection of PPD (Rich, 1951).

The question arises whether TBC-induced inhibition of adjuvant arthritis may depend on a mechanism similar to that mediating the immune deviation described by Asherson and Stone (1965). These authors showed that the immune response to an antigen administered to guinea-pigs in Freund’s complete adjuvant was modified by injecting the same antigen as an aqueous preparation beforehand. This led to a reduction in delayed-type sensitivity and a qualitative alteration in the antibody produced. The effect was still apparent if the modifying injection was delayed until the day after the injection of antigen in complete Freund’s adjuvant, but not if it were delayed until the sixth day. Crowle and Hu (1966) have described a somewhat similar modification of the immune response (split tolerance) seen in mice after the injection of antigen in a water-in-oil emulsion. This could be achieved by aqueous preparations of the antigen administered, not only beforehand but up to as long as 2 weeks after the definitive challenge.

The present experiments did not include the administration of intraperitoneal TBC before inducing arthritis, but Gery and Waksman (1967) have shown that pre-treatment with a course of TBC-insulin injections effectively prevents arthritis appearing, although it does not prevent the PPD skin test becoming positive. Interestingly, these pre-treated rats will develop arthritis if injected with lymphocytes from appropriately sensitized donors, but if they themselves receive the sensitizing injection, their lymphocytes will not produce arthritis when injected into normal recipients.

Another experimental disease which has been successfully suppressed by the administration of antigen is allergic encephalomyelitis. Administration of brain tissue to rats either during the neonatal period (Paterson, 1958) or to adult rats receiving cyclophosphamide (Salvin and Liauw, 1968) induces a state of tolerance to the responsible antigen, which protects the animals against a subsequent encephalitogenic challenge. More relevant to the present work is the observation by Alvord, Shaw, Hruby, and Kies (1965) that this disease can be prevented or suppressed by injection of nervous tissue (particularly as a water-in-oil suspension) given either before or after the encephalitogenic challenge. Even when injections were delayed until clinical signs had appeared, there was some suppression. It is of interest that, in this better defined model, the inhibition of encephalitis correlated with a decrease in the delayed skin response and an increase in the serum antibody titre to nervous tissue. However, the mechanism which brings about these changes is still uncertain.

The significance to the clinician of studies such as these are obviously the therapeutic possibilities which they raise. The inhibition of experimental encephalomyelitis might, for example, be relevant to the treatment of demyelinating diseases. The inhibition of adjuvant arthritis described here is a reminder that, in the rheumatological field also, administration of specific antigen may offer an approach to the suppression of immune-mediated disease.
Summary

Adjuvant arthritis in the rat was inhibited by the intraperitoneal injection, during the incubation period, of 100 mcg. or more of dead tubercle bacilli in suspension.

By contrast, tuberculin (PPD) in doses of 4 to 100 mcg. accentuated the lesions. Pertussis vaccine, aluminium hydroxide gel, and sheep erythrocytes produced less marked and inconsistent effects.

It is suggested that the inhibitory effect of mycobacteria injected in this way may be due to the peritoneal cavity providing a sump in which specifically instructed immunocytes are trapped, leaving insufficient numbers to mount a reaction elsewhere.

Discussion

DR. J. S. LAWRENCE (Manchester) Have you tested the rat sera for the presence of rheumatoid factor?

DR. CURREY No. I have carried out sheep cell tests in another series of rats with adjuvant arthritis and they were all negative. Since sheep cells are so useful for tagging things on, there are data on various indirect agglutination tests of that type.

DR. D. L. GARDNER (London) The only matter on which I would disagree is that a therapeutic lesson for human disease can safely be taken from rat adjuvant arthritis. The question remains whether the effect demonstrated today is immunologically specific or whether it is the result of non-specific reaction mediated by the large surface of the peritoneum. Thus, an injection of alcohol will have the same effect.

DR. CURREY The evidence in this case comes mainly from the specificity, and of course I have tested only a limited number of substances. These include a number of experiments in which sheep cells have been given as primary or secondary challenge. We have also injected other adjuvants—pertussis organisms and aluminium hydroxide-gel—these did not show similar effects, so to some extent it seems to be specific. It might be argued that we have not tested anything so irritant in the peritoneum as whole tubercle.

DR. D. L. GARDNER (London) Did you try re-injection to see if the suppression was lasting or temporary?

DR. CURREY I have not tried that. On the whole animals in whom we produce arthritis and then challenge again do not get further arthritis. We have not challenged these later on to see if we could produce it.

I think it may be relative. This is a very critical model. The balance is easily upset, probably depending on a critical and transient relationship between delayed hypersensitivity and antibody production.

DR. TALAL (Bethesda) It would be very interesting to see if you could transfer the arthritis by removing the lymphoid cells by lymphatic duct drainage and putting them into other animals.

DR. CURREY This is an experiment that should be done. We have found the transference of adjuvant arthritis to be very difficult.

DR. P. J. L. HOLT (London) Is the whole tubercle bacilli soluble? PPD is soluble, and this difference in solubility might explain the different effects.

DR. CURREY This is quite true, the protein purified derivative is soluble.

DR. B. M. GREENWOOD (Taplow) I should like to support Dr. Currey's view of the delicate balance present in the adjuvant arthritis model. We have found that injection with an avirulent strain of malaria parasite inhibits adjuvant arthritis, while a virulent strain, which stimulates the reticuloendothelial system, does not.

References


Asherson, G. L., and Stone, S. H. (1965) Immunology, 9, 205 (Selective and specific inhibition of 24 hour skin reactions in the guinea-pig. 1. Immune deviation: Description of the phenomenon and the effect of splenectomy).


Résumé

La modification de l'arthrite provoquée par l'adjuvant chez le rat par les injections intrapéritonéales de mycobactéries complets morts ou de tuberculin

L'arthrite provoquée par l'adjuvant chez le rat était inhibée par l'injection intrapéritonéale de 100 mcg. ou plus de bacilles tuberculeux morts et en suspension faite pendant la période d'incubation.

Par contraste, la tuberculine en doses de 4 à 100 mcg. avait accentué les lésions. Le vaccin contre la coqueluche, l'hydroxide d'aluminium gel et les érythrocytes du mouton produisaient des effets inconsistants et moins marqués.

Il est suggéré que l'effet inhibiteur de mycobactéries injectées de cette façon peut être dû à ce que la cavité péritonéale fournit un réservoir dans lequel des immunocytes spécifiquement choisis sont bloqués laissant ainsi un nombre insuffisant pour commencer une réaction ailleurs.

Sumario

Modificación de la artritis adyuvante en la rata por inyecciones intraperitoneales de microbacteria muerta o de tuberculin

La artritis adyuvante en la rata fue inhibida, durante el periodo de incubación, mediante la inyección intraperitoneal de 100 mcg. o más de bacilo tuberculoso muerto, en suspensión.

Por contraste, la tuberculina (PPD) en dosis de 4 a 100 mcg. acentuó las lesiones. La vacuna de tos ferina, la gel de hidróxido de aluminio y los eritrocitos de oveja produjeron efectos menos marcados e inconsistentes.

Se sugiere que el efecto inhibitorio de microbacteria inyectada de este modo quizá se deba a que la cavidad peritoneal ofrece un pozo colector en el cual quedan atrapados inmunocitos específicamente aleccionados, dejando libres cantidades insuficientes para producir una reacción en otro punto.
Adjuvant arthritis in the rat. Effect of intraperitoneal injections of either whole dead mycobacteria or tuberculin.

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