Effect of excising Freund's adjuvant granuloma on subsequent development of experimental allergic arthritis

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SUMMARY Rabbits were immunized with antigen in Freund's complete adjuvant. Several weeks later the granuloma which developed was excised one day before joint challenge with antigen. The subsequent development of experimental allergic arthritis (EAA) was not affected, which argues against the chronicity of the disease being maintained by continuous recruitment of mycobacterial debris to EAA joints.

The injection of a soluble, protein antigen into the joints of rabbits previously immunized with that antigen in Freund's complete adjuvant (FCA) results in chronic persisting arthritis—experimental allergic arthritis (EAA) which histologically resembles rheumatoid arthritis (Dumonde and Glynn, 1962; Consden *et al.*, 1971). One explanation for the chronicity of the disease is the possibility that there is a continuous recruitment of macrophages carrying mycobacterial debris, from the FCA granuloma (which develops at the immunization site) to EAA joints. Here it might be released where it could cause tissue damage in its own right or encourage autoimmunization, in either event resulting in further attraction of macrophages.

We present evidence that surgical removal of the FCA granuloma one day before joint challenge with antigen does not affect the subsequent development of the disease, which argues against continuous recruitment of mycobacterial debris to EAA joints.

Materials and methods

Rabbits were of the Old English strain bred in the animal house of the MRC Rheumatism Unit, Taplow. Freund's incomplete adjuvant (FIA) was purchased from Difco Laboratories, East Molesey, Surrey. Freeze dried *Mycobacterium tuberculosis* heat killed strains C, DT, and PN mixed was supplied by the Central Veterinary Laboratory, Weybridge, Surrey.

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IMMUNIZATION SCHEDULE

Eighteen rabbits were given a subcutaneous injection of an emulsion consisting of equal parts of FCA and saline containing 10 mg ovalbumin (OA) per ml. 4 weeks later 10 of the animals were anaesthetized by intravenous injection of Nembutal, and 1.0 ml of 1/10 000 dilution adrenaline diluted in 10.0 ml lignocaine was injected subcutaneously. A single incision was made and all the granuloma visible to the naked eye removed. The site was then closed with interrupted sutures. The following day a solution of 10 mg OA in 1.0 ml sterile saline was injected into the left knee joint of all 18 rabbits. The left and right joints were measured regularly for 8 weeks (one animal was killed at 10 days owing to excessive chewing at its left joint) when the animals were killed and left and right joints removed for histology. The subcutaneous operation site was also examined for presence of granuloma material.

Results

Preliminary experiments in which synovectomies were carried out on joints which had established EAA (in some cases with additional granulomec-tomies) proved to be extremely traumatic, and many of the animals had to be killed as a result of self injury by chewing at the operated joint. This approach was therefore abandoned. Granulomec-tomies alone proved to be mild and no animals were lost as a result of the operation. Examination of the operation site at autopsy did occasionally show the presence of small pieces of granuloma, but the vast majority were certainly removed.

JOINT MEASUREMENTS

All injected joints were extremely swollen at 24 hours, which indicated a strong immune response. However, by the close of the experiment knee swellings had decreased appreciably in both groups (see Fig.), and the mean swelling in the two groups was of similar magnitude.

HISTOLOGY

Both groups of animals developed EAA as judged by histological examination of joint sections (Table).

Fig. Mean joint swellings in rabbits after joint challenge. Swelling is expressed as the difference in diameter between the injected (left) and noninjected knees.

Table Degree of arthritis in two groups of rabbits with experimental allergic arthritis. Group I had had the subcutaneous granuloma surgically removed one day before joint challenge with antigen, group II had no surgery

Grade of arthritis	Group I	Group II
3+	2	1
2+	0	2
1+	4	3
±	3	1
0	1	0

Discussion

Animals developed EAA (as judged by joint swellings and histological examination of autopsy specimens) whether the subcutaneous granuloma had been removed one day before intra-articular injection of antigen or not, indicating that continuous recruitment of mycobacteria (from the subcutaneous granuloma) is not an important factor in the pathogenesis of EAA. However, it should be stressed that a small amount of granuloma may not have been removed, and some adjuvant material may have disseminated before surgery. Similar results were obtained on adjuvant arthritis by Ward and Jones (1962) who found that removal of the adjuvant depot more than 2 hours after injection did not affect the subsequent development of allergic arthritis in rats. Furthermore we have already provided strong, but not conclusive, evidence that neither live *M. avium* nor [¹²⁵I] labelled dead *M. tuberculosis* are carried to joints of animals with EAA from FCA granuloma depots (Doble *et al.*, 1975).

We therefore conclude that continuous recruitment of mycobacterial debris from FCA granuloma depots to EAA joints is not an important factor in the chronicity of EAA.

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