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Experimental Immune Inflammation in the Synovial Membrane

I. THE IMMUNOLOGICAL MECHANISM

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Summary. Synovitis was produced in immunized guinea-pigs and rats by injection of antigen into the knee joint. The reaction was mainly mononuclear at 48 hours and, in the guinea-pig, progressed to chronic granuloma formation. Antigens principally used were bovine γ-globulin and tuberculin PPD. Immune deviation, giving a diminished delayed hypersensitivity response, also gave diminished synovial inflammation when compared with undeviated control animals. Immune synovitis to tuberculin PPD was successfully transferred with peritoneal cells taken from immunized animals and given intravenously to normal recipients whose knee joints were injected with antigen. Intravenous transfer of immune serum gave rise to a synovial reaction to bovine γ-globulin, injected into recipients' joints. Transfer of both cells and serum gave rise to particularly severe reactions. Transfer of either cells or serum or cells with serum failed to give reactions extending beyond 3–4 days. This suggests that active immunization is a requisite for the chronic inflammatory reaction of synovitis. Although the 48-hour synovial membrane reaction of rats was similar to that seen in the guinea-pig, chronic inflammatory reactions were not found in that species.

INTRODUCTION

Experimental arthritis is a well-established model for the study of inflammation (Dumonde and Glynn, 1962). In an immunized animal this can readily be evoked by injecting the appropriate antigen into the knee joint. This approach has been used in the present experiments, which were primarily designed to discover the relative roles of circulating antibody and of delayed hypersensitivity (cellular immunity) in the mediation of the inflammatory lesion of the synovium. At the same time, the work provided an opportunity to study extracutaneous delayed hypersensitivity. Cellular immunity has usually been studied by skin reaction; however, Coe and Feldman (1966) described its occurrence in the guinea-pig bladder, and Schlossman and Stetson (1957) have studied the corneal reaction in delayed hypersensitivity in the guinea-pig.

In the present study several different approaches were used. Antigens, such as tuberculin PPD or azo-benzene arsanilic acid were used to give preferentially cellular immune reactions, and bovine γ-globulin to give both antibody and delayed hypersensitivity. The

system of immune deviation of delayed hypersensitivity as studied by Asherson and Stone (1965), and Loewi, Holborow and Temple (1966), was used to give a diminished delayed hypersensitivity reaction, in the presence of antibody. Cells, serum, or both were transferred from immunized donors to recipients which were then challenged by antigen injection into the knee joints. The synovial membrane was found to be suitable for the demonstration of a cellular immune reaction, but a serum antibody reaction could also be demonstrated. Guinea-pigs were used in most of the experiments, since delayed hypersensitivity has been most extensively studied in this species. In some of the work involving cell transfer a strain of inbred rats was used.

MATERIALS AND METHODS

Outbred guinea-pigs of the Hartley strain, weighing 350–500 g were used. Rats came from the local laboratory Wistar colony or from a locally maintained colony of inbred August rats. Weights ranged from 200 to 300 g.

Animals were immunized by intracutaneous injection of antigens with complete Freund's adjuvant. Amount of antigen usually employed was 100 μg/animal. Bovine γ-globulin (BGG) was purified by column chromatography on DEAE-cellulose. Azobenzene-arsonate (ABA) was prepared according to the method of Leskowitz (1962). This was coupled to BGG or to guinea-pig albumin (GPA) by diazotization. Immune deviation was achieved as previously described (Loewi *et al.*, 1966) by immunization with an intravenous dose of 1 mg alum-precipitated PPD (tuberculin purified protein derivative) or BGG, followed 1 week later by 1 mg BGG with complete adjuvant intracutaneously.

Cells were transferred from immunized donors. For this, paraffin oil was introduced into the peritoneum of donors and removed with washing 4 days later. Cells were centrifuged, washed and re-suspended. Viability assessed by eosin exclusion was found to be >90 per cent. Between 1 and 3×10^8 cells were transferred to recipients intravenously and antigen injected into a skin site or into a knee joint immediately. Cells were also obtained from lymph nodes and spleens of immunized animals by mincing with scissors, followed by aspiration of the fluid phase obtained by adding medium Parker 199. Cells were washed, centrifuged and re-suspended and finally injected intravenously into recipients.

Sterile test antigen was injected into knee joints 2–3 weeks after immunization. BGG was heated at 65° for 20 minutes to achieve aggregation. Tuberculin PPD was obtained from Evans Medical Ltd. Half a milligram in 0·1 ml of these or of ABA–BGG or ABA–GPA was injected into the knee joint, or 10 μg into a skin test site in the guinea-pig flank or 25 μg in the rat's ear. In the latter case the result was assessed by pre- and post-injection measurements of ear thickness. Observations were made at 4, 24 and 48 hours. Polylysine hydrobromide (430 residues) was used at 500 μg/0·1 ml as a non-specific inflammatory agent in some experiments.

At varying intervals, the animals were killed and knee joints opened. The synovium of the infrapatellar pad was dissected from the patellar tendon and processed for histology.

Tanned cell haemagglutination was performed according to the method of Stavitsky (1954), complement fixation by the method of Donnelley (1951), and passive cutaneous anaphylaxis by the method of Ovary and Bier (1954), allowing 18 hours for antibody fixation in the skin.

RESULTS

1. RESPONSE IN THE SYNOVIAL MEMBRANE OF IMMUNIZED ANIMALS

(a) *Guinea-pigs*

Synovial membrane taken 48 hours after an intra-articular challenge in a total of forty immunized guinea-pigs showed signs of inflammation. The stroma beneath the layer of synoviocytes showed varying numbers of cells, most of which could be described as histiocytes or macrophages. The nature and origin of these cells will be considered in detail in a succeeding paper. In addition there was a variable number of polymorphs. Typical small lymphocytes were rarely seen. The surface layer of synoviocytes showed no

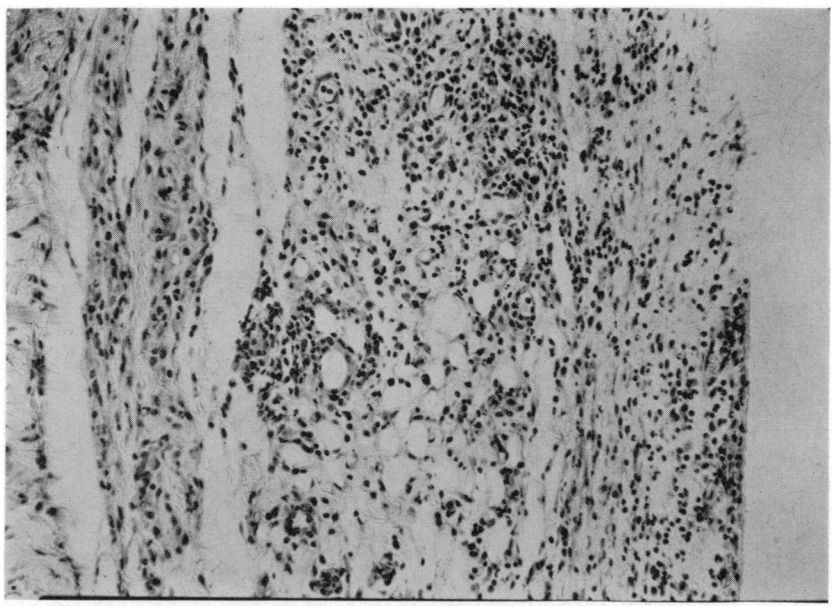

FIG. 1. Synovial membrane of immunized guinea-pig, 48 hours after intra-articular injection of 500 μg BGG into knee joint. The stroma shows invasion by mononuclear cells. H & E, $\times$ 105.

significant changes by light microscopy. In animals immunized with BGG and complete adjuvant, BGG injected into one knee, and PPD into the other gave similar histological appearances (Fig. 1). Synovia were also taken at longer intervals following knee joint challenge (forty guinea-pigs). At 10–20 days, in many experiments (Figs. 2 and 3) but not in every case, chronic inflammation was still present with, in some cases, new formation of connective tissue, large numbers of fibroblasts, and occasional small nests of what appeared to be small lymphocytes. Plasma cells were seen in small numbers. Some hyperplasia of surface synoviocytes was present, but this was not a prominent feature. In control experiments in which BGG or PPD was injected into the knee joints of twenty unimmunized animals the synovial membrane showed no reactions to BGG, and only very occasional small cellular infiltrates in response to PPD at 48 hours. No reactions were seen after longer intervals.

The tuberculin reaction is usually accepted to be due to cellular immunity, without a significant contribution from circulating antibody, and in the above experiments antibody

to tuberculin PPD could be demonstrated in only a few instances by the passive cutaneous anaphylaxis reaction. The experiments with ABA were undertaken because ABA is an

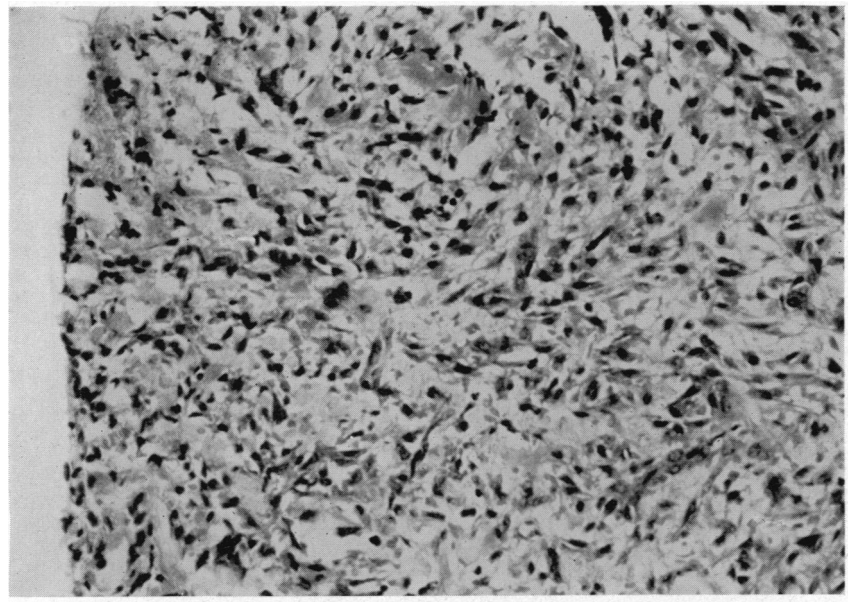

FIG. 2. As Fig. 1, synovial membrane 9 days after BGG challenge. A granuloma consisting of mononuclear cells with fibril formation is present. H & E, ×105.

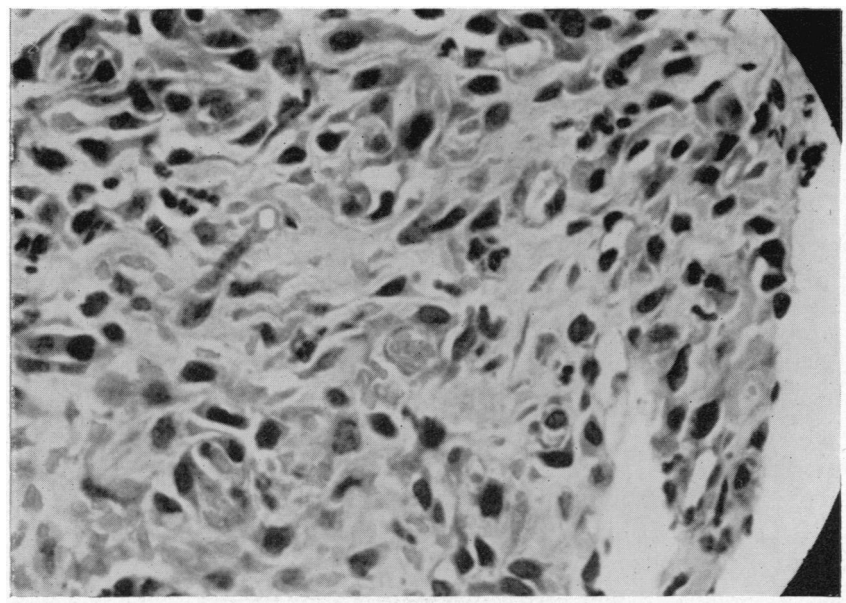

FIG. 3. As Fig. 1, synovial membrane 13 days following BGG challenge. Mononuclear cells, mostly of the macrophage type, are seen. H & E, ×315.

antigen considered (Leskowitz, 1962) to call forth only a reaction of delayed hypersensitivity in the guinea-pig. It was found that animals immunized with azo-benzene-

arsonate *N*-acetyl tyrosine, and showing delayed hypersensitivity reactions of the skin when challenged with 10 μg ABA–GPA showed only low-grade inflammatory reactions in the joint synovia when challenged intra-articularly with 500 μg ABA–GPA or ABA–BGG. Thus, although both tuberculin PPD and ABA consistently gave delayed hypersensitivity reactions in the skin of suitably immunized animals, a strong inflammatory response in the synovium was evoked only by PPD, and little inflammation resulted when ABA was injected into the joint.

The effect of immune deviation was assessed in twelve guinea-pigs, six of which were given a preliminary intravenous injection of alum-precipitated PPD, and six others an intravenous injection of alum-BGG. Subsequently all were immunized with BGG and complete adjuvant intracutaneously. Two days after skin testing and obtaining a blood sample, the animals were injected with BGG in the right knee joint and with PPD in the

TABLE 1

THE EFFECT OF IMMUNE DEVIATION ON IMMUNE INFLAMMATION ON GUINEA-PIG SYNOVIAL MEMBRANE

Pre-immunization	Skin reaction		Thickness increase	Synovial reaction		
	Antigen	Diameter		Antigen	2 days	13 days
BGG	BGG	8·2 mm (0–12)	14·0% (0–48)	BGG	2 nil, 1+	2 nil, 1+
	PPD	14·5 mm (14–16)	38·7% (0–100)	PPD	3++	2+, 1++
PPD	BGG	15·8 mm (13–22)	51·2% (13–70)	BGG	1+, 2++	1 nil, 1+, 1++
	PPD	12·7 mm (10–14)	18·2% (0–27)	BGG	2+, 1++	3 nil

Figures for skin reactions are means, with range in parentheses, of 24-hour reactions in each group of three animals. + indicates an inflammatory reaction of moderate degree, ++ one of great intensity and extent.

left. Three of the animals pre-immunized with PPD, and three pre-immunized with BGG were killed 2 days after knee injection, and the rest at 13 days. Table 1 shows that animals that had been pre-immunized showed diminished 24-hour skin reactions, and tended also to show a less severe inflammatory joint reaction than controls. Other experiments along these lines showed similar results. In the case of PPD, the reduced response may confidently be ascribed to diminished cellular hypersensitivity, but in the case of BGG it has to be remembered that as well as diminished delayed hypersensitivity reactions, immune-deviated animals show qualitative alterations of circulating antibody (Loewi *et al.*, 1966). Quantitatively however, in the experiment shown in Table 1, tanned-cell haemagglutination titres for antibody to BGG were similar in the deviated and the control group, as were the complement fixation titres, which varied between 1:4 and 1:16.

(b) *Rats*

Eighteen rats were immunized with BGG and complete Freund's adjuvant intracutaneously. Knee joints were injected with BGG or PPD 10–14 days later. Synovial membrane, removed 48 hours later, regularly showed inflammatory changes. In contrast to the findings in guinea-pigs, however, when the knee joints were studied 9–18 days following intra-articular antigen challenge, no or only slight histological signs of synovial inflammation were found in twelve rats.

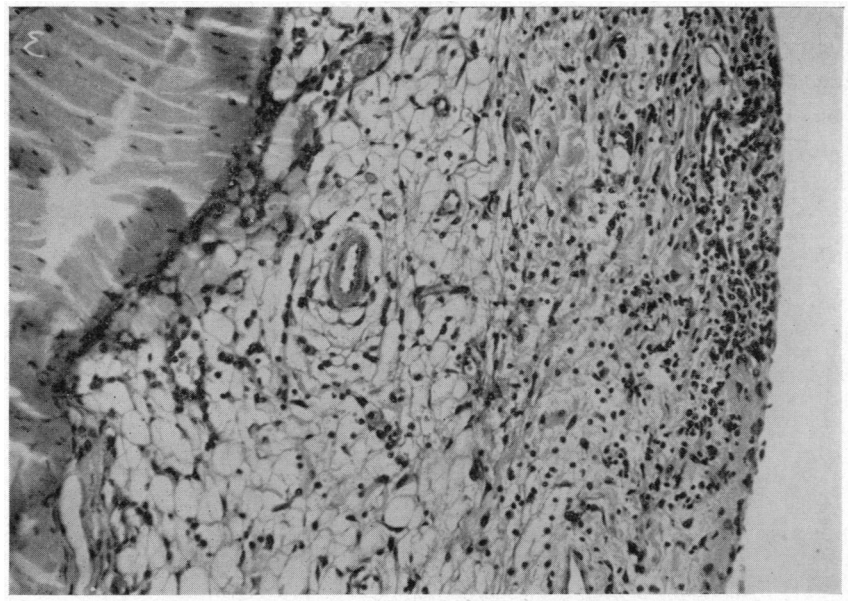

FIG. 4. Synovial membrane of rat, 48 hours after intravenous transfer of 10^8 peritoneal cells from immunized donors, and tuberculin PPD injection into the knee joint. Cellular infiltration is present beneath the surface synovial layer. H & E, $\times$ 105.

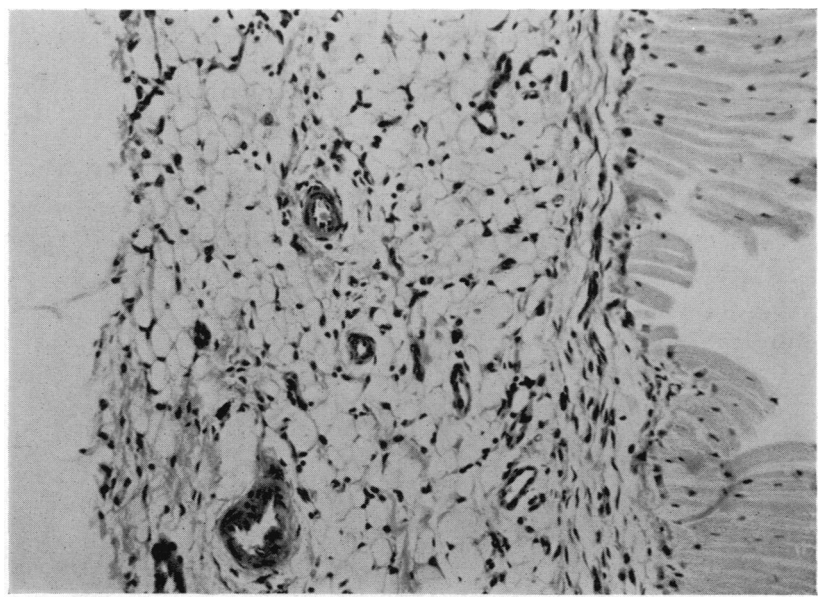

FIG. 5. Rat synovial membrane taken 9 days after peritoneal exudate cell transfer by intravenous route and intra-articular PPD. Few signs of inflammation present. H & E, $\times$ 105.

2. TRANSFER EXPERIMENTS

(a) *Cell transfer*

The most direct proof of the role of cellular immunity can be obtained by the transfer of cells from an immunized donor to an unimmunized recipient, followed by appropriate testing of the latter. Peritoneal exudate cells were obtained from guinea-pigs immunized with BGG and complete adjuvant, so that the recipients could be tested with both BGG and PPD. In guinea-pigs, transfer of approximately 10^8 peritoneal exudate cells, 70–90 per cent of which appeared morphologically to be macrophages, resulted in inflammatory responses to PPD in the synovia of five of seven recipients 48 hours after transfer and challenge. Transfer followed by skin testing was similarly successful. No evidence for the induction of chronic synovial inflammation by transfer of cells was obtained. With BGG, on the other hand, a moderately strong inflammatory transferred synovial response occurred in only one of five recipient guinea-pigs.

Six guinea-pigs received transfers of between 3 and 6×10^8 spleen or lymph node cells from immunized donors. In four of these, a slight inflammatory response to intra-articular PPD was obtained, while BGG gave an equivocal inflammatory response in three recipients. Six other recipients, given spleen or lymph node cells at the same time, and subjected to skin tests likewise showed only weak equivocal responses consisting of slight erythema without induration to BGG or PPD. Transfer of peritoneal cells was thus unequivocally successful, while the response to lymph node or spleen cell transfer was largely negative.

Peritoneal exudate cells were also transferred between inbred August rats, each rat receiving 10^8 cells, intravenously. The donors were immunized with Freund's complete adjuvant. Skin tests performed in the ears of three recipient rats using 25 μg PPD, gave strongly positive 24-hour reactions, with an increase in ear thickness of 100 per cent or more. Three other recipients were given challenges with PPD in the knee joint. In two animals killed at 48 hours, there was a strong inflammatory synovial reaction (Fig. 4). The third animal was examined at 9 days, when only remnants of chronic inflammation were seen (Fig. 5).

(b) *Serum transfer with or without cells*

The role of serum antibody in immune synovitis was examined in a group of eight guinea-pigs. They received an intravenous injection of 5–8 ml of serum, obtained from guinea-pigs which had been immunized with BGG and complete adjuvant. The pooled serum gave reactions of precipitation and complement fixation with BGG. The synovial membrane of four animals examined 48 hours after transfer and challenge showed an intense inflammatory haemorrhagic reaction (Fig. 6). However, four others taken 19 days after transfer and challenge, were normal (Fig. 7), with the exception of a little surface hyperplasia. Finally, a group of six guinea-pigs received 2×10^8 peritoneal exudate cells together with 5 ml guinea-pig pooled anti-BGG serum intravenously. The right knee joint was injected with 500 μg BGG and the left with 500 μg PPD immediately afterwards. Examination of two recipients 48 hours later showed an intense inflammatory reaction of the BGG-injected synovial membrane (Fig. 8), with a moderate inflammation of the PPD side. The effect of the transfer of cells and serum was to produce an inflammation to BGG greatly in excess of that seen in guinea-pigs by cell transfer only and apparently more intense than that given by serum transfer. A third recipient showed extensive

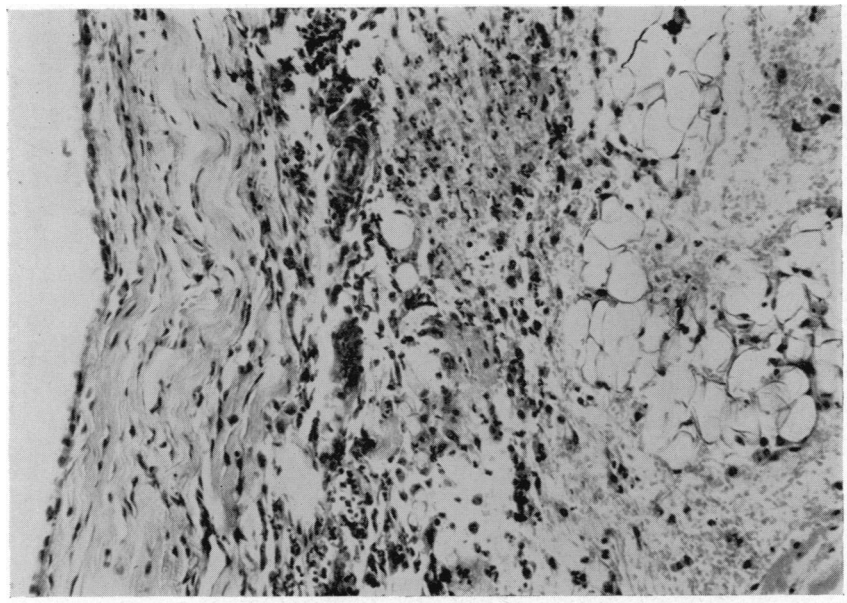

FIG. 6. Synovial membrane of guinea-pig, 48 hours after intravenous transfer of anti-BGG serum and BGG injection into knee joint. Lesion is seen deeply in the tissue. It consists of some necrosis and haemorrhage. Polymorphs and mononuclear cells are seen. H & E, × 105.

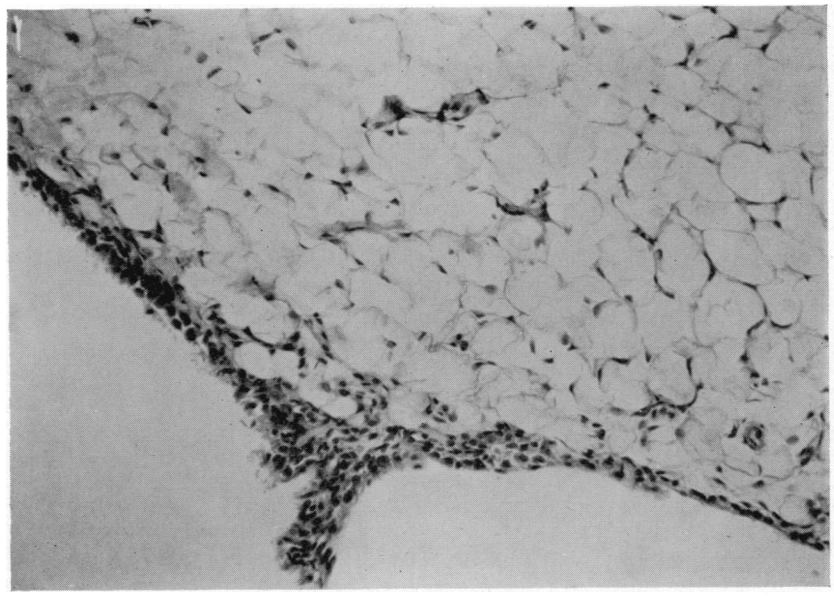

FIG. 7. Synovial membrane of guinea-pig 10 days after intravenous anti-BGG serum and intra-articular BGG challenge. No signs of chronic inflammation in synovial stroma. H & E, × 105.

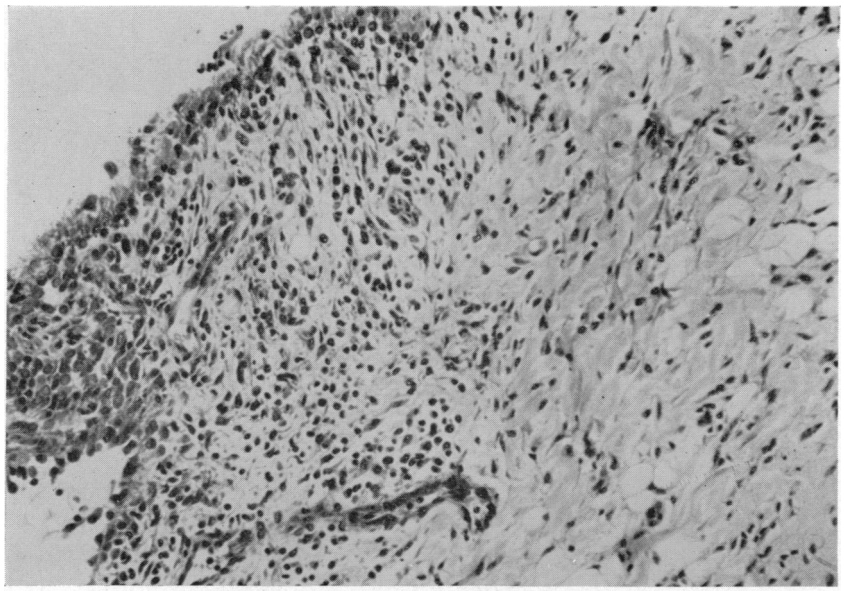

FIG. 8. Synovial membrane of guinea-pig which had 4 days previously received 10^8 peritoneal exudate cells and serum from immunized donors and received BGG in the knee joint. There is mononuclear cell infiltration in the synovial stroma. H & E, × 105.

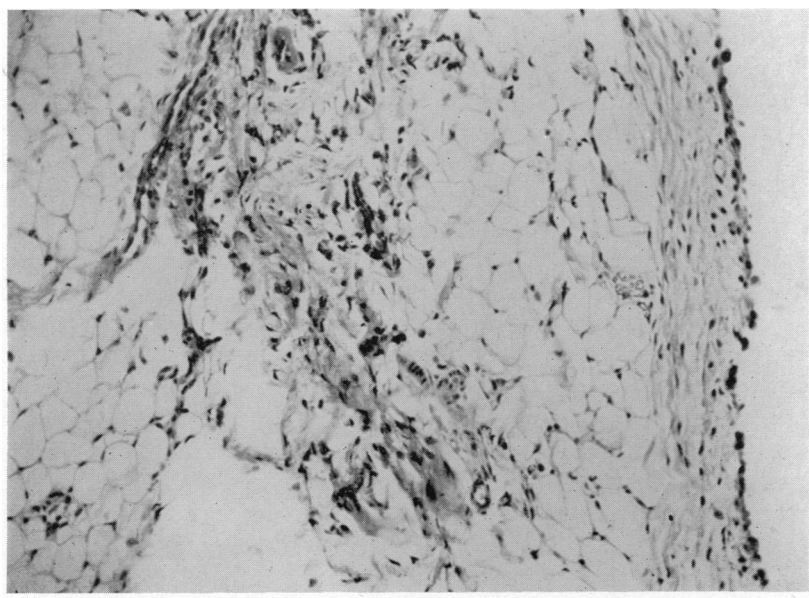

FIG. 9. Guinea-pig synovial membrane 14 days after peritoneal cell and serum transfer intravenously from immunized donors and intra-articular BGG challenge. There are few signs of inflammation. H & E, × 105.

inflammation of the BGG synovium at 4 days, with a moderate reaction of the PPD-injected side. Two other guinea-pigs examined 8 and 14 days (Fig. 9) after transfer and challenge, showed only some remnants of inflammation. These data show that transfer, at least in outbred guinea-pigs, although successful in the first instance does not lead to a state of chronic inflammation.

DISCUSSION

The work presented shows that a reaction of delayed hypersensitivity can take place in the synovium of an immunized animal. The evidence for this is successful transfer by cells, the reaction to tuberculin PPD, and the effect of immune deviation. Other extra-cutaneous sites in which a reaction of delayed hypersensitivity has been shown are the guinea-pig cornea, and the guinea-pig bladder. That this role for cellular immunity in immune synovitis is not exclusive is apparent from the experiments involving serum transfer, when a severe reaction to BGG could be demonstrated in synovium. It further appeared that some enhancement of the reaction could be obtained by the concurrent transfer of cells and serum antibody to BGG. Such a synergic effect of cells and serum has been shown for the skin reaction by Asherson and Loewi (1966). While in some systems, including that of immune orchitis (Brown, Glynn and Holborow, 1967), cellular immunity and serum antibody appear to act in synergy, in others such as immune encephalitis (Paterson, 1961), antagonism has been shown in that antibody has been found to protect against the apparently cell-mediated lesion. From the present work it appears that the lessened delayed hypersensitivity response occasioned by immune deviation resulted in a diminished synovial response. Similar findings have been reported by Jankovic and Flax (1963) for experimental guinea-pig thyroiditis. Pre-immunization without adjuvant resulted in diminished delayed hypersensitivity reactions as well as in a reduced incidence of histological evidence of thyroiditis.

The histology of the synovial reactions up to 3 weeks from the date of challenge in the knee joint showed a predominantly histiocytic picture resembling the skin reaction seen at 24–48 hours. Polymorphs were rarely prominent in these lesions, while plasma cells and typical small lymphocytes only made a late appearance on the scene. The cell population and its origin and fate will be the subject of a separate paper.

The lesions induced in the synovial membrane of actively immunized guinea-pigs almost invariably progressed to a chronic stage, in contrast to the transferred lesions with serum, or cells and serum, or with cells, even in inbred animals; such transfers, although giving positive results 48 hours after challenge, did not progress to chronicity. It would appear, therefore, that a site of immunization presumably in association with its draining sensitized lymph nodes, is necessary in guinea-pigs for the synovial lesion to progress. The function of the lymph nodes may eventually be taken over on a local basis when lymphoid foci develop in the synovium as shown by Glynn (1966) in the rabbit, and more speculatively, as frequently seen in the synovium in rheumatoid arthritis.

In experiments in rats immunized with BGG with complete adjuvant, I have only rarely found chronic synovial lesions. This may be associated with the rapid healing of the immunization site in this species, and be reflected in the difficulty with which delayed hypersensitivity to BGG or PPD is transferred in rats (personal unpublished observations).

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