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Joint inflammations and flare-up reactions in mice induced by a helper T cell clone

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Abstract

Joint inflammation was induced in mice by cloned helper T cells specific for methylated bovine serum albumin (mBSA). This occurred after local injection of the helper T cells together with mBSA into the knee joint, but also when the helper T cells were intravenously injected and the antigen directly into the joint.
Local injection of mBSA several weeks after waning of a joint inflammation induced by cloned helper T cells caused a flare-up reaction, indicating that the helper T cells persisted in the joint after the primary inflammation.

Introduction

The antigen induced arthritis (AIA) is a model system in which animals are immunized with antigen emulsified in complete Freunds adjuvans. Direct injection of the antigen into the knee joint of the animal 2 or 3 weeks after immunization evokes an inflammation which resembles the inflammation in the human rheumatoid joint.
Methylated bovine serum albumin (mBSA), which has a positive net charge because of the methylation, is a suitable antigen in this model. This antigen is retained in the cartilage structures, which have a negative charge. The retention of the antigen in the joint is considered to be important for chronicity of the inflammation [1].
In the AIA model it has been shown that it is also possible to induce flare-up reactions after the inflammation has waned. This was achieved by repeating local administration of the antigen in the knee joint, but also by intravenous (iv) or oral administration of the antigen [2, 3].
Rheumatoid arthritis (RA) shares some characteristics with delayed type hypersensitivity (DTH) reactions [4]. As clones of helper T cells can induce DTH reactions in mice [5], we studied whether they can also induce joint inflammation. For this purpose we used a T cell clone directed against mBSA. We also investigated whether such an inflammation induced by cloned helper T cells, after waning, can be induced to flare-up again by injection of the antigen only.

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Materials and methods

Mice

Female C57BL/6J mice were purchased from OLAC Ltd., Bicester, U.K. They were used at the age of 2–3 months.

Cloned helper T cells

A T cell clone directed against mBSA was made according to procedures described elsewhere [6]. The clone was cultured in serum-free medium (IMDM-ATL) [7]. Every 5–6 weeks the clone was restimulated with the antigen (5 μg/ml mBSA). After three days the restimulated T cells were transferred to medium containing 600 U/ml recombinant IL-2 which was a kind gift of Dr. J. Besemer from Sandoz Ltd., Vienna, Austria.

Induction of arthritis and induction of flare-up reactions

Arthritis was induced in two ways. Firstly by intra-articular (ia) injections of 60 μg mBSA or ovalbumin (OVA) (both Sigma Chemical Company, St. Louis, Missouri, U.S.A.) into a hind knee joint, 0.5 to 1 hour later followed by ia injection of the cloned helper T cells into the same joint. Secondly by iv injection of the helper T cells followed by injections of the antigen ia. Flare-up reactions were induced by local administration of 60 μg antigen 1.5–3 weeks after induction of the joint inflammation.

^{99m}Tc-uptake measurements

Measurement and quantitation of joint inflammation was performed by the ^{99m}Tc-uptake method. This method has been described in detail elsewhere [8]. The severity of inflammation was expressed as the specific increase of knee thickness and was calculated as [(uptake right knee-uptake left knee)/uptake left knee] × 100%.

Histology

Knee-joints of both knees were removed *in toto*, fixed in 10% phosphate-buffered formalin and decalcified in 5% formic acid. Standard frontal sections (6 μm) were prepared of paraffin-way embedded knee-joints and stained with haematoxylin and eosin.

Results

We investigated whether cloned helper T cells specific for mBSA can induce joint inflammation. The reaction was quantitated by ^{99m}Tc uptake 24 hours after the clone was injected into the right hind knee joint together with the mBSA. As a control similar helper T cells were injected together with the non-crossreacting antigen OVA in the left hind knee. After ia injection of 3×10^5 mBSA specific cloned T cells and 60 μg mBSA a clear joint inflammation was found (Fig. 1, upper bar). Local reinjection of mBSA 12 days later caused a flare-up reaction (Fig. 1, middle bar). For comparison, the lower bar in Fig. 1 shows the response of mice 12 days after a single injection of mBSA specific helper T cells and mBSA, which is the smouldering chronic phase of the inflammation. Joint inflammation could also be induced by iv injection of cloned mBSA specific helper T cells and ia injection of mBSA only. Fig. 2 (upper bar) shows the specific increase in knee thickness after iv injection of 1.5×10^6 cloned helper T cells. When the mice were challenged 3 weeks later by another ia injection of the same antigen, a flare-up reaction was found (Fig. 2, middle bar).
The inflamed joints of the mice used for Fig. 2 were also examined histologically.

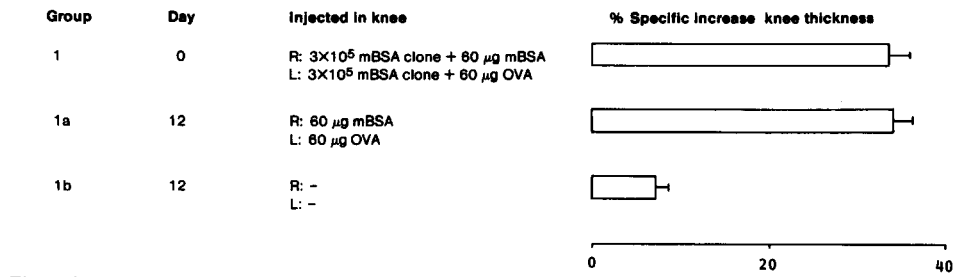

Figure 1
Quantitative inflammation measurements, expressed as specific increase of ^{99m}Tc uptake (mean ± SEM) in knee joints of mice with an arthritis induced by local injection of a cloned helper T cells specific for mBSA. 1: n = 8 1a: n = 7 1b: n = 5.

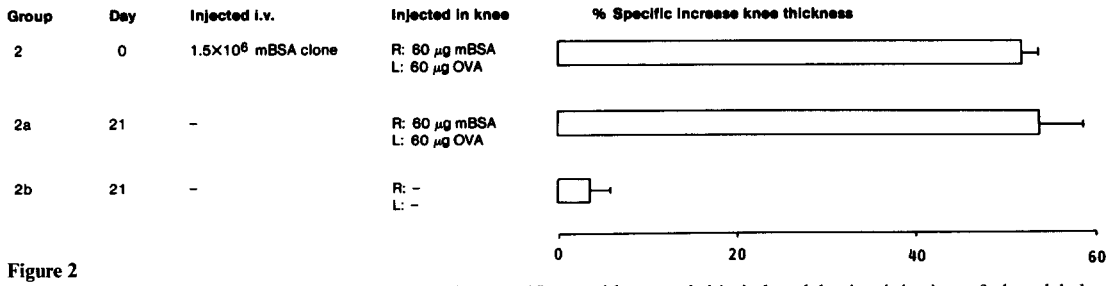

Figure 2
Quantitative inflammation measurements, expressed as specific increase of ^{99m}Tc uptake (mean ± SEM) in knee joints of mice with an arthritis induced by i v injection of cloned helper T cells specific for mBSA. 2: n=12 2a: n=4 2b: n=3.

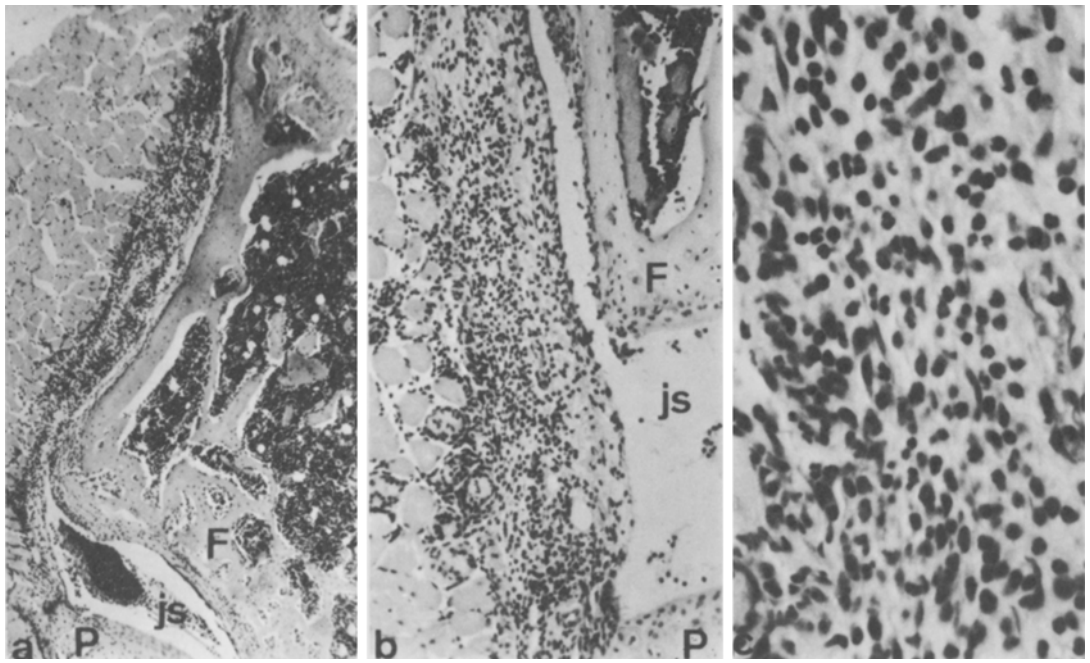

Figure 3
a. Arthritis 24 hours after induction by iv injection of mBSA specific helper T cells and local injection of the antigen (HE. 5.4×) b. flared arthritis 24 hours after local rechallenge with the antigen of situation a. (HE, 14×) c. Detail of b. which shows the polymorphonuclear infiltrate (HE, 54×). P. is patella, F is femur, js is joint space.

In the right hind knee 24 hours after induction of the inflammation an extensive inflammation was seen with large numbers of granulocytes and a small number of lymphocytes and macrophages.
In the left hind knee of the same mice only a small infiltrate with some oedema and some granulocytes was observed.
The infiltrate found during the flare-up reaction was comparable to the one seen 24 hours after the first inflammation was induced. In the control group, where no flare-up reaction was induced, foci of inflammation were seen and a higher percentage of monocytes along with some granulocytes.

Discussion

The data presented here show that joint inflammation can be induced in mice by cloned mBSA specific T cells with the helper phenotype.

This inflammation could be induced by local injection of the T cells in combination with ia injection of the mBSA, but also by iv injection of the T cells in combination with ia injection of the mBSA. The latter method is of course more physiological and therefore a better model for studies on joint inflammation.

The inflammation induced by cloned mBSA specific helper T cells is chronic. Evidence for the chronicity of the inflammation stems from the observation that flare-up reactions can be induced by local challenge with the antigen only. This flare-up reaction cannot be attributed to an immunization effect, because two ia injections of 60 μg mBSA two weeks apart only led to a minor specific Tc uptake.

Histologically a clear inflammatory reaction was seen in joints of mice injected with the cloned mBSA specific T cells. This was the case after local as well as after iv injection of the cloned T cells. The inflammation did not differ from an inflammation induced in the AIA model. Both have characteristics of a DTH reaction.

Human rheumatoid arthritis and AIA both show aspects of DTH. In the AIA model flare-up reactions can be induced analogous to the exacerbations seen in RA [2]. Here we show that similar joint inflammation can be induced by cloned helper T cells, indicating that a disease like RA might be initiated by T cells alone [9].

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