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Antigen-induced arthritis: inflammation and antigen handling after two different doses of intra-articularly injected antigen

H. J. VAN BEUSEKOM, L. B. A. VAN DE PUTTE, W. B. VAN DEN BERG & W. J. M. VAN DEN BROEK* *Department of Rheumatology and *Isotope Laboratory of the Department of Internal Medicine, St. Radboud Hospital, University of Nijmegen, The Netherlands*

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Summary. We studied (i) the inflammatory response induced by intra-articular (i.a.) injection of 2·5 and 0·5 mg ^{125}I-labelled bovine serum albumin (^{125}I-BSA) into the paired knee joints of rabbits immunized with BSA in Freund's complete adjuvant, and (ii) handling of these different doses of antigen by means of regular external radioactivity measurements. The joint inflammation was quantified by ^{99m}Technetium pertechnetate uptake measurements. More severe arthritis was seen in the 2·5 mg BSA-injected than in the paired 0·5 mg BSA-injected knee, both in the early and in the late phase of antigen-induced arthritis. External radioactivity measurements showed enhanced disappearance of radioactivity from the 2·5 mg ^{125}I-BSA challenged knee joint, resulting in lower percentual retention of the dose injected than in the 0·5 mg injected knee joint. However the calculated absolute amount of long term retained BSA in the 2·5 mg BSA injected knee, 4 weeks after i.a. injection, was still about 2·8 times the quantity of BSA retained in the 0·5 mg BSA-injected paired knee joint. These data indicate that the severity of both the early and late phase of antigen-induced arthritis is i.a. antigen dose-dependent and, in addition, suggest that handling of i.a. antigen depends in part on the severity of joint inflammation.

Correspondence: Dr H. J. van Beusekom, Department of Internal Medicine, St. Radboud Hospital, University of Nijmegen, The Netherlands.

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INTRODUCTION

Antigen-induced arthritis (AIA), firstly described by Dumonde & Glynn (1962), has been widely used as a model for the study of human rheumatoid arthritis in view of similar histopathological features and its chronicity. The dose of intra-articularly (i.a.) injected antigen in this model appears to be important, since it has been shown that, in ovalbumin immunized rabbits, only i.a. doses of more than 10 μg of the antigen resulted in histological signs of inflammation of the synovial membrane (Consden, Doble, Glynn & Nind, 1971). In addition, studies on the effect of varying doses of i.a. antigen injected repeatedly at short intervals on the resulting joint inflammation, suggest that, in the dose range studied, the largest doses of i.a. antigen are associated with the most severe inflammatory phenomena (Goldlust, Rich & Brown, 1978). Dose–response studies on the effect of varying single doses of i.a. antigen on the severity of AIA have not been published, probably because they are hampered by the great inter-individual variation in immune and inflammatory responses in outbred species like rabbits (Menard & Dion, 1975) and also by methodological difficulties in quantification of joint inflammation.

This report deals with quantification of inflam-

mation in the early and in the later phase of AIA in rabbits after i.a. injection of two different doses of antigen into both knee joints of the same animal, thus avoiding the problem of inter-individual variations in immune and inflammatory responses. Joint inflammation was measured by the ^{99m}Technetium pertechnetate uptake method, a reliable method to detect and quantify joint inflammation in rabbits (van Beusekom, van den Broek, van de Putte, Buijs & van den Berg, 1981a). Since the severity of joint inflammation may influence handling of i.a. antigen (van Beusekom, van de Putte, van den Berg, van den Broek & Buijs, 1981b), we studied the latter by regular external radioactivity measurement of radiolabelled i.a. antigen.

MATERIALS AND METHODS

Animals

New Zealand white rabbits of either sex, weighing between 2·5 and 3·5 kg and aged 3–5 months were used. They were obtained commercially, caged individually, fed a commercial pellet diet supplemented with furazolidone (100 mg/kg), oxytetracycline (150 mg/kg) and sulphadimidine (100 mg/kg) and given water *ad libitum*.

Materials

Bovine serum albumin (BSA, lot number A-4378 Sigma, Chemical Company, St. Louis, Mo.), Freund's complete adjuvant (FCA, containing mycobacterium strain H37RA) were used. ^{125}I-iodine carrier and reductant-free was obtained from Radiochemical Centre, Amersham.

Iodination of BSA

^{125}I labelling of BSA was performed by the chloramine T method (Hunter & Greenwood, 1962). ^{125}I-labelled BSA (^{125}I-BSA) was separated from free ^{125}I by Sephadex G25 fractionation and stored at −20°. Refractionation of unfrozen material, to be used for i.a. injection, revealed more than 99% of the radioactivity to be in the protein fraction.

Immunization schedule and arthritis induction

Seven rabbits were immunized with 5 mg BSA in FCA and skin tested with 100 μg BSA 3 days before i.a. antigen injection. Three weeks after immunization the right knee joint was injected with 2·5 mg BSA and the left knee with 0·5 mg BSA. In five out of seven rabbits the i.a. injected BSA was trace-labelled with ^{125}I-BSA. The dose of radioactivity of ^{125}I applied to both knee joints was similar, 8 μCi. Lugol's solution was orally administered from 3 days before until 1 week after i.a. antigen injections to minimize ^{125}I uptake by the thyroid gland. After the period studied, the rabbits were further used for experiments studying the influence of systemically injected homologous antigen on the ongoing arthritis (van Beusekom *et al.*, manuscript in preparation).

Measurement of antigen retention

Antigen retention was determined by external radioactivity measurement of the knee joint as described (van Beusekom *et al.*, 1981b) on consecutive days. The radioactivity measured is expressed as percentage of the count rate immediately following i.a. injection of ^{125}I-BSA (percentage antigen retention). The quantity of BSA retained in the knee joint was estimated from the percentage of the radioactivity still present and the dose of i.a. injected BSA. All measurements were corrected for physical decay.

Measurement of inflammation of the knee joint

Joint inflammation was assessed by ^{99m}Technetium pertechnetate uptake measurements ($^{99m}TcO_4$ uptake), as described before (van Beusekom *et al.*, 1981a). $^{99m}TcO_4$ uptake measurements were performed 2, 4, 7, 14 and 24 days after i.a. antigen injection in seven rabbits and once more 49 days after i.a. injection in four rabbits. The increase of $^{99m}TcO_4$ uptake was related to the median of three $^{99m}TcO_4$ uptake measurements of the same knee joint before arthritis induction (ΔTc uptake). Δ Tc uptake values of 28·0 c.p.m./μCi, or more, indicate joint inflammation, as previously reported (van Beusekom *et al.*, 1981a). Swelling of the knee joint was determined by diameter measurements with an engineer's micrometer.

Measurement of serum anti-BSA

BSA antibody titres were determined by the haemagglutination of BSA-coated sheep erythrocytes prepared with the chromium chloride method (Sweet & Welborn, 1971). Sera were obtained before i.a. antigen challenge and frozen at −20°.

Statistical calculations

Statistical calculations were performed by the paired Student's *t* test (two tailed).

RESULTS

$^{99m}TcO_4$ uptake and diameter measurement

$^{99m}TcO_4$ uptake measurements were performed before and at various intervals after i.a. injection of 2·5 mg BSA into the right and 0·5 mg into the left knee joint of seven immunized rabbits (Fig. 1). One rabbit died of pneumonia 3 days after i.a. injections. Two days after i.a. injections, in the acute phase of arthritis, both knee joints of all rabbits showed enhanced $^{99m}TcO_4$ uptake as compared with the $^{99m}TcO_4$ uptake of the knee joints before induction of arthritis (ΔTc uptake). Both right and left knees exhibited a ΔTc uptake exceeding 28 c.p.m./μCi which was previously described as indicating joint inflammation (van Beusekom *et al.*, 1981a). The 2·5 mg BSA-injected right knees showed a significantly higher ΔTc uptake than the paired left knees in six out of seven rabbits, compatible with more pronounced acute joint inflammation. In the remaining rabbit the ΔTc uptake was equal for both knee joints. After day 2 ΔTc uptake values exceeding 28 c.p.m./μCi persisted in the right knee for the time studied. The small dose injected paired left knees showed ΔTc uptake values not always exceeding 28 c.p.m./μCi. Of a total of thirty-five paired ΔTc uptake values, thirty showed higher values in the right knee than in the left knee.

An increase in joint diameter by 1 mm or more was seen 2 days after i.a. injection in six right knees and five left knees of seven rabbits. In five rabbits the diameter of the right knee exceeded the diameter of the paired left knee by 1 mm or more. Twenty-eight days after i.a. injection the diameter of the right knee remained increased in five out of six rabbits, but only three rabbits showed a persistent increase in diameter of the left knee. A difference in diameter of the right knee as compared with the left knee of 1 mm or more existed at this moment in no more than two rabbits. When looking for a correlation between ΔTc uptake values and serum anti-BSA haemagglutination titres, no correlation could be found.

External measurements of radiolabelled antigen

The radioactivity measurements over the knee joints of five rabbits, expressed as percentage antigen retention, on consecutive days after i.a. injections, are shown in Table 1. Two days after i.a. injections the radioactivity of the 2·5 mg ^{125}I-BSA injected right knee joints exceeded that in the paired 0·5 mg ^{125}I-BSA injected left knees in three out of five rabbits whereas two rabbits showed the reverse. Thereafter, all left knees showed a slower decrease in radioactivity than the paired right knees, resulting in a significant

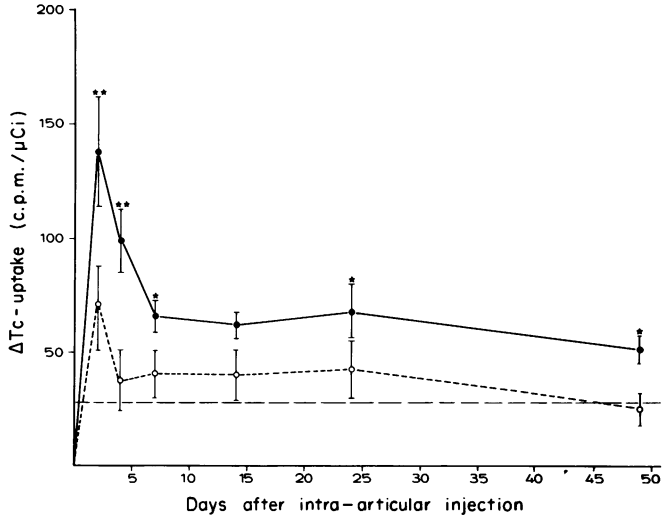

Figure 1. ΔTc uptake measurements (see Materials and Methods) on consecutive days after arthritis induction with 2·5 mg (●) and 0·5 mg (○) bovine serum albumin injected intra-articularly into the paired knee joints of six rabbits. ΔTc uptake values in the figure represent mean values. Vertical bars show standard error of the mean. Significance of paired differences are indicated by * $P<0·05$; ** $P<0·01$. The interrupted line represents the upper range of the normal variation of ΔTc uptake values (van Beusekom *et al.*, 1981a).

Table 1. Retention of BSA in the knee joints at various intervals after intra-articular injection of 2·5 mg [^{125}I]-BSA into the right knee and 0·5 mg ^{125}I-BSA into the left knee in immunized rabbits

Days after i.a. injections	R. knee (2·5 mg)		L. knee (0·5 mg)			R:L ratio‡	
	%	μg	%	μg	*P* value§	%:%	μg:μg
2*	4·08	102	3·72	19	NS	1·06	5·3
4	0·95	24	1·23	6·2	NS	0·78	3·9
7	0·38	10	0·66	3·3	<0·01	0·60	3·0
9	0·33	8·2	0·56	2·8	<0·005	0·58	2·9
11	0·29	7·2	0·51	2·6	<0·01	0·57	2·8
14	0·25	6·2	0·44	2·2	<0·01	0·56	2·8
18	0·21	5·2	0·39	2·0	<0·05	0·54	2·7
24	0·18	4·5	0·31	1·6	<0·01	0·57	2·8
28	0·16	4·0	0·28	1·4	<0·001	0·57	2·8
35†	0·21	5·2	0·34	1·7		0·61	3·0
42†	0·21	5·2	0·34	1·7		0·59	3·0

* Group of five rabbits.
† Group of two rabbits.
All data represent mean values.
‡ Mean of R:L ratios of the knee joints of individual rabbits.
§ Statistically significant paired differences of the percentage BSA retention of 2·5 mg and 0·5 mg injected knee joints calculated with the Student's *t* test.
NS, no significant difference.

difference of radioactivity between the knees from 7 days after i.a. injections until the end of the study. However, the calculated quantity of BSA retained in the right knee joints remained 2·8 times the quantity BSA retained in the left paired knee joints (Table 1).

In conclusion the large dose (LD)-challenged knee, showing the highest ΔTc uptake values, had the lowest longterm percentage antigen retention as compared with the small dose (SD)-injected knee joint.

DISCUSSION

This study shows that in the presence of identical systemic immunity, a large dose of i.a. antigen results in more severe acute and chronic joint inflammation than a small dose in the dose range tested. These results were obtained by using a sensitive method to detect and quantify joint inflammation, $^{99m}TcO_4$ uptake measurement (van Beusekom *et al.*, 1981a). and by studying the inflammatory effect of both a LD and a SD of i.a. antigen in each individual animal, thus eliminating inter-individual variations in immune and inflammatory responses innate to outbred species. From day 2 to day 7 after i.a. antigen injection, disappearance of radioactivity, and therefore the percentage elimination of antigen, from the LD joint exceeded that from the SD joint. Thereafter it was identical for both joints. At any given moment after i.a. antigen injection, however the absolute amount of antigen in micrograms in the LD joint exceeded that in the SD joint.

Although AIA has been widely used as a model for human rheumatoid arthritis, only scanty data are available on factors determining the severity of acute and chronic joint inflammation after i.a. injection of the antigen. Several studies (Glynn, 1968; Goldberg, Lance & Davis, 1974; Menard & Dion, 1975) have indicated that adequate cell-mediated immunity to the antigen used is essential for induction of chronic arthritis, and it has been suggested that, the better the cell-mediated immunity, the less i.a. antigen is needed for arthritis induction and *vice versa* (Page-Thomas, 1977). However, to our knowledge no quantitative data have been published on this matter. Even less is known of the role of humoral immunity. Intra-articular injection of antigen in the mere presence of humoral immunity, as found after immunization with Freund's incomplete adjuvant containing the antigen, only results in a transient Arthus-like type of inflam-

mation (Glynn, 1968). The importance of humoral immunity in the presence of adequate cell-mediated immunity for the severity and chronicity of joint inflammation is not clear. It probably plays a role in the chronicity of antigen-induced arthritis (Cooke & Jasin, 1972a; Menard & Dion, 1975), since it largely determines prolonged antigen retention in the joint (Hollister & Mannik, 1974; Jasin, 1975; Menard & Dion, 1976) a phenomenon held responsible for the protracted course of this type of joint inflammation (Consden *et al.*, 1971; Webb, Ford & Glynn, 1971; Cooke, Hurd, Ziff & Jasin, 1972b; Hollister & Mannik, 1974; van Beusekom *et al.*, 1981b). In addition, correlations have been found between serum antibody titres and the severity of AIA several weeks after i.a. antigen challenge, graded by a histological score (Cooke & Jasin, 1972a; Menard & Dion, 1975). These correlations, however, may merely reflect the fact that the degree of delayed-type hypersensitivity determines both the severity of joint inflammation (Goldberg *et al.*, 1974; Menard & Dion, 1975) as well as the antibody response (Cooke & Jasin, 1972a; Menard & Dion, 1975). In our study we found no such correlation, using $^{99m}TcO_4$ uptake measurement as quantitative measure of joint inflammation, neither in the early phase nor in the late phase of AIA. Admittedly, numbers of animals studied were small.

To our knowledge no studies have been published on the effect of different single i.a. doses of antigen on the resulting severity of AIA. The principal reason for this is probably the fact that, until recently, no reproducible method of quantification of rabbit arthritis was available (van Beusekom *et al.*, 1981a). However, studies varying the number and magnitude of i.a. antigen challenges suggest that repeated LD of i.a. antigen result in a more severe and destructive type of arthritis than SD. (Goldlust *et al.*, 1978). Our data indicate that this also applies to single i.a. doses of antigen, not only in the early acute but also in the chronic phase of AIA.

A similar antigen dose dependency of immune inflammation has previously been demonstrated under certain conditions in skin tests in guinea-pigs (Baer & Kolb, 1967; Jokipii & Jokipii, 1973). We have taken due note of another finding, obtained in studies using skin tests as a model of inflammation, that may relate to our data. It has been shown that simultaneous skin tests in delayed-type hypersensitivity suppress each other under certain conditions (Baer & Kolb, 1967; Jokipii & Jokipii, 1973; von Blomberg, Boerrigter & Scheper, 1978), whereas this does not apply to Arthus-type skin tests (von Blomberg *et al.*, 1978). Baer and Kolb (1967) have suggested that the suppressive effect is especially important when simultaneous doses of antigen are used that differ more than three- to four-fold, causing the SD skin test almost to disappear. There are, however, several possible reasons why this suppressive phenomenon has not interfered to a notable degree with our studies. Firstly, the early acute phase of AIA is largely an Arthus-type of inflammation, which is not sensitive to inflammation suppression (von Blomberg *et al.*, 1978). Secondly, inflammation measurements of both inflamed knees parallelled each other, not only in the early phase but also, with almost similar differences, thereafter. In addition $^{99m}TcO_4$ uptake values in this study of bilateral arthritis were comparable with those found after unilateral arthritis induced with similar doses of BSA (van Beusekom, unpublished results). We cannot, however, exclude minor influences of one joint inflammation on the other.

The data available show that LD i.a. injected antigen is handled differently by the joint than SD i.a. injected antigen. This difference in antigen handling occurred in the first week after i.a. antigen injection, resulting in a lowering of right:left (R:L) ratios of external radioactivity measurement (Table 1), which indicates that a higher percentage of antigen disappeared from the LD than from the SD joint. The explanation for this phenomenon may be the difference in inflammatory activity between the joints, indicating more pronounced vascular phenomena and more phagocytosis in the LD joint. After the first week R:L ratios of external radioactivity measurements become almost constant, indicating that identical percentages of antigen disappear from each joint. In this phase of joint inflammation, the bulk of retained antigen is probably tightly bound to avascular and hypovascular joint structures (Cooke *et al.*, 1972b; Hollister & Mannik, 1974) and this may explain the almost constant R:L ratios of external radioactivity measurement, although distinct differences in inflammatory activities between these joints still exist.

Our data indicate that the severity and duration of AIA depend in part on the dose of i.a. antigen and in addition, that inflammatory phenomena in the joint influence antigen handling. Therefore, further studies on antigen handling in the joint should include data on these aspects. The meaning of these data remains a matter of speculation. Therapeutic efforts in human chronic immune arthritis, e.g. rheumatoid arthritis, have so far been aimed at modulation of immune and

inflammatory responses. Our data suggest that, once putative antigens are implicated, it may be worthwhile to focus on therapeutic modalities that reduce the amount of antigen entering the joint.

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