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# Allergic arthritis induced by cationic antigens: relationship of chronicity with antigen retention and T-cell reactivity

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## SUMMARY

In order to define the antigenic properties necessary for sustained allergic arthritis, we prepared a range of differently charged bovine serum albumin (BSA) species with increasing isoelectric points (4·5, 4·5–7·4, 7–8, 8·5–9 and >9). The highly cationic BSA >9 appeared to be in a polymeric form. We investigated three properties of these proteins: (i) antigen retention, (ii) T-cell reactivity, and (iii) arthritis induction. Injection of the respective radiolabelled antigens in the knee-joints of immunized mice showed that antigen retention increased with cationicity of the proteins, with the best retention found for BSA with pI > 9. However, sustained joint inflammation was only found with $BSA_{8\cdot5\text{–}9}$, and not with a level BSA of lower or even higher pI. T-cell reactivity *in vivo* as measured by delayed-type hypersensitivity (skin testing) was similar for the tested antigens, with the exception of polymeric BSA (>9). The latter appeared to be a poor antigen. *In vitro*, T-cell reactivity ([$^3$H]-thymidine incorporation) against the cationized BSA species was slightly higher as compared to native BSA. The combination of excellent antigen retention and adequate T-cell reactivity appears to be optimal for the induction of chronic arthritis.

## INTRODUCTION

Chronic allergic arthritis can develop upon the administration of an antigen into the joint of previously immunized animals. This model for rheumatoid arthritis was described in the rabbit using BSA (Cooke & Jasin, 1972) or ovalbumin (OA) (Consden *et al.*, 1971) as the antigen. In mice, native BSA fails to induce arthritis (van den Berg *et al.*, 1984). Recent studies in our laboratory showed that highly positively charged BSA [isoelectric point (pI 8·5] causes severe, long-lasting arthritis (van den Berg *et al.*, 1984).

For chronic arthritis, two mechanisms seem to be important: sufficient retention of the antigen in the joint structures, and a good T-cell response directed against the antigen. A high isoelectric point of a protein leads to a tremendous increase of association of the protein, with both cartilage and surrounding tissues compared to anionic proteins (van den Berg *et al.*, 1984; Schalkwijk *et al.*, 1985; van Lent *et al.*, 1987). Physicochemical properties of the joint structure rich in negatively charged proteoglycans are probably responsible for this phenomenon (van den Berg *et al.*, 1986).

Apart from retention of the antigen, a sufficient T-cell response against the antigen is a prerequisite for arthritis development and chronicity (Brackertz, Mitchell & Mackay, 1977). T-cell deficient nu/nu mice were not susceptible to arthritis induction with methylated BSA.

The objectives of the present study were as follows: firstly to delineate the exact isoelectric point of an antigen necessary for optimal induction of a long-lasting arthritis, and secondly to characterize the contribution to chronicity of inflammation of two determinants, antigen retention and cellular immunity. To this end we prepared a range of BSA derivatives with different isoelectric points and determined the following parameters: arthritis induction in mice, antigen retention *in vivo* and *in vitro*, and antigen-induced proliferation of splenic T lymphocytes.

Abbreviations: BSA, bovine serum albumin; DTH delayed-type hypersensitivity; EDC 1-ethyl-3(3-dimethyl-aminopropyl carbodiimide); ELISA, enzyme-linked immunosorbent assay; pI, isoelectric point; PBS, phosphate-buffered saline.

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## MATERIALS AND METHODS

### *Animals*

Male C57BL mice aged 8–10 weeks at the start of the immunization were used.

### *Antigens*

Bovine serum albumin (fraction V) and 1-ethyl-3(3-dimethyl-aminopropyl)carbodiimide hydrochloride (EDC) were obtained from Sigma Chemical Company, St Louis, MO. *N, N-*

Dimethyl-1, 3-propanediamine (DMPA) was obtained from BDH Chemicals Ltd, Poole, Dorset, U.K. Anionic proteins were transformed to cationic derivatives according to the method of Danon *et al.* (1972) using EDC as activator and DMPA as a nucleophile. Briefly, 1·25 ml DMPA in 10 ml PBS was adjusted with 1 N HCl to pH 5·0. In addition, 500 mg BSA and increasing amounts of EDC (100, 250, 350 and 1000 mg) were added. After stirring for 4 hr the solution was dialysed against PBS and the modified proteins were freeze-dried. In this way free carboxyl groups of the protein are coupled to amino groups of DMPA. The pI of the protein will thus be raised, since anionic groups are eliminated and cationic groups are introduced by DMPA. Cationic polymers were made by raising the EDC concentration (1000 mg) and purified by gel filtration on Sephadex G-200.

*Protein characterization*

The isoelectric points of the various proteins were determined in a 5% polyacrylamide slab gel with 0·8% ampholines (pH gradient from 3·5 to 9·5). Sodium dodecyl sulphate–polyacrylamide gel electrophoresis (SDS–PAGE) was performed according to Laemli (1970). Proteins were separated on a 7–18% gradient polyacrylamide slab gel to check the purity of the preparations.

*$^{125}I$ labelling*

This was performed by the Chloramine T method (Hunter & Greenwood, 1962). $^{125}$I-labelled proteins were separated from free $^{125}$I by Sephadex G-25 gel filtration.

*Immunization*

Mice were immunized with 0·1 ml antigen in Freund's complete adjuvant emulsion (Difco Laboratories, Detroit, MI) by injections into the flank skin and the footpads of the forelegs. Heat-killed ($2 \times 10^9$) *Bordetella pertussis* organisms (National Institute of Public Health, Bilthoven, The Netherlands) were administered intraperitoneally as an additional adjuvant. Booster injections were given subcutaneously on Day 7.

*Skin testing*

Arthus reaction and delayed hypersensitivity were measured at 4 and 24 hr, respectively, after injection of 5 μg antigen in 10 μl saline into the pinna of the ear. Increases in ear thickness were measured with an engineers micrometer.

*Antigen retention measurements*

$^{125}$I-labelled antigen (60 μg) was injected in the right knee-joint, whereas the left knee-joint received saline. At various days thereafter (see results), the $^{125}$I radioactivity of both knees was measured by external gamma counting. Values of the right knee were corrected for that of the left knee, which represents background activity. Antigen retention in the knee-joint was expressed as a percentage of the initial count rate measured immediately after antigen injection.

*Arthritis induction*

Immunized mice were injected intra-articularly in the knee joint with 60 μg antigen in 6 μl PBS. At Days 3, 7 and 14, groups of mice were anaesthesized by ether inhalation.

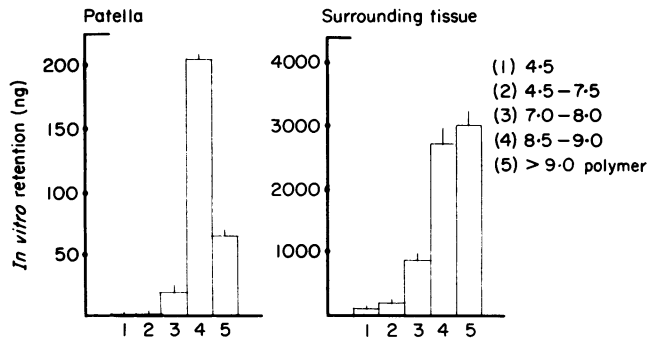


**Figure 1.** *In vitro* retention of radiolabelled monomeric BSA with variable isoelectric points and cationic polymeric BSA in murine patella and surrounding tissue. Values represent the mean ± SD of groups of

*$^{99m}Tc$ uptake measurements*

Joint inflammation was determined by $^{99m}$Tc pertechnetate uptake measurements of the knee-joint. This method makes use of the increased accumulation of a systemically administered small isotope at the inflamed site, which is due to augmented blood flow and tissue swelling. The method has been described in detail elsewhere, and $^{99m}$Tc-uptake values correlate well with histological parameters of joint inflammation (Kruijsen *et al.*, 1981)

*Antigen retention* in vitro

Patellae with surrounding tissue were removed from the knee-joint of normal C57BL mice as previously described (van den Berg *et al.*, 1982). The patellae with adjacent tissue were incubated in microtitre plates in 100 μl phosphate-buffered saline containing 30 μg protein (3 μCi/μg) for 2 hr at 37°. Subsequently the patellae were washed exhaustively in PBS, fixed in 2% glutaraldehyde in PBS, and decalcified in 5% formic acid. After this procedure the patella is easily separated from the surrounding tissue. The amount of protein retained in patellar cartilage and surrounding tissue was quantified by gamma counting and expressed as nanograms per patella.

*Autoradiography*

Dry deparafinized tissue sections of whole patellae were covered by a photographic emulsion (K5 emulsion, Ilford Ltd, Mobberley, Cheshire, U.K.). After exposure the emulsion was developed with Kodak developer D19 and fixed in 24% sodium thiosulphate.

*Antibody determination*

Antibodies were measured with an ELISA assay. Antigen was coated on microtitre plates (Greiner, Alphen a/d Rijn, The Netherlands) at a concentration of 100 μg/ml. Antibody titres were assessed by two-fold serial dilution of the sera followed by detection of bound mouse Ig with 1:500 diluted peroxidase-conjugated rabbit anti-mouse Ig (Miles Laboratories Inc., Elkhart, IN). 0-Phenylenediamine (1 mg/ml; Sigma) was used as substrate for peroxidase, and the antibody titre was determined by using 50% of the maximal extinction as an end-point.

*T-cell proliferation assay*

Mouse spleen cells were isolated and washed in RPMI supplemented with 10% human serum, glutamin (2 mM) and pyruvate

(1 mM). Erythrocytes were lysed by treatment of the cells with an 0·16 M $NH_4Cl$ solution in 0·17 M Tris, pH 7·2, for 5 min. After two washes in RPMI, the cells were plated on plastic T flasks (75 $mm^2$) from Falcon Plastics, Oxnard, CA. After 60 min of incubation at 37°, the non-adherent cells were harvested by aspiration and two 4·5-ml RPMI washes of the adherent cells. The harvested cells were then passed over nylon-wool columns. Before use the columns were washed with PBS and RPMI. The cells were incubated at 37° on the column for 45 min. At the end of the incubation the non-adherent cells were eluted from the column with 10 ml of warm RPMI. After this, 100 $\mu$l of RPMI containing $1 \times 10^5$ T-cell enriched spleen cells were placed in each well of a sterile, U-bottomed, polystyrene microculture plate (Costar, Cambridge, MA). Antigens or mitogens were added in another 100 $\mu$l to give a total volume of 200 $\mu$l, and final concentrations of antigen of 25, 12·5, 2·5, 0·5 and 0·1 $\mu$g/ml. Cultures were maintained at 37° in a humified atmosphere of 2% $CO_2$ and 98% air for 5 days. Sixteen hours before harvesting, 1 $\mu$Ci of [$^3$H]-thymidine (6·7 Ci/mmole from New England Nuclear, Boston, MA) was added in 25 $\mu$l of RPMI. Cultures were harvested on filter paper strips with a Titertek cell harvester 550, washed with distilled water and then fixed with 96% ethanol. The filter discs for each sample were placed in 0·5 ml of scintillation fluid, and the radioactivity was monitored in a liquid scintillation counter (Beckmann Instruments, Palo Alto, CA). All determinations were done in quadriplate, and the data are expressed in mean counts per minute ± the standard deviation.

## RESULTS

### Protein modification and characterization

The isoelectric point of a native protein can be raised by amidation. By increasing the EDC concentrations, we made BSA (monomer) derivatives with varying isoelectric points. In the presence of high concentrations of EDC, high molecular weight cationic polymers of BSA are obtained. These complexes were purified by G-200 gel filtration. Complexes with a molecular weight of about 450,000 were used in our experiments. The charge and the molecular weight of the native and modified antigens were characterized by isoelectrofocusing and SDS–PAGE. The isoelectric points of the monomeric preparations were 4·5, 4·5–7·4, 7–8 and 8·5–9, respectively, whereas the polymeric form showed an isoelectric point higher than 9.

### Protein retention *in vitro*

Using these well-defined BSA species, protein retention at joint structures was measured *in vitro* using a dissected murine patella. After incubation with radiolabelled proteins and washing of non-bound BSA label, the patella was separated from the surrounding tissue, and the amount of $^{125}$I-radiolabelled proteins retained in patellar cartilage and surrounding tissue was measured separately by gamma counting. The data, depicted in Fig. 1, demonstrate that BSA retention in patellar cartilage dramatically rises at an isoelectric point of 8·5–9. The highly cationic polymeric BSA showed a moderate retention. Autoradiography studies demonstrate that the polymeric proteins are not able to penetrate the dense cartilage matrix but only stick to the surface, whereas the monomeric cationic species penetrate deeply into the cartilage (data not shown). In the surrounding tissue the amount of retained protein also increases with higher isoelectric points, but the change is more gradual.

### Protein retention *in vivo*

In order to investigate the retention *in vivo*, we injected 60 $\mu$g of $^{125}$I-labelled proteins with various isoelectric points into the knee-joint of non-immune mice. Radioactivity was measured at various time-intervals by external gamma counting. Only small amounts of native BSA and $BSA_{4.5-7.4}$ were retained in the joint (Fig. 2a). The more cationic monomeric derivatives show a higher retention. $BSA_{8.5-9}$ persisted about three times better in the joint than $BSA_{7-8}$. The highly cationic polymeric derivative showed the highest retention of all preprations tested. Figure 2b shows the retention pattern upon injection in immunized mice. A similar pattern of increase in retention in parallel with increases in pI was noted in these animals, but over the whole range the retention was somewhat less.

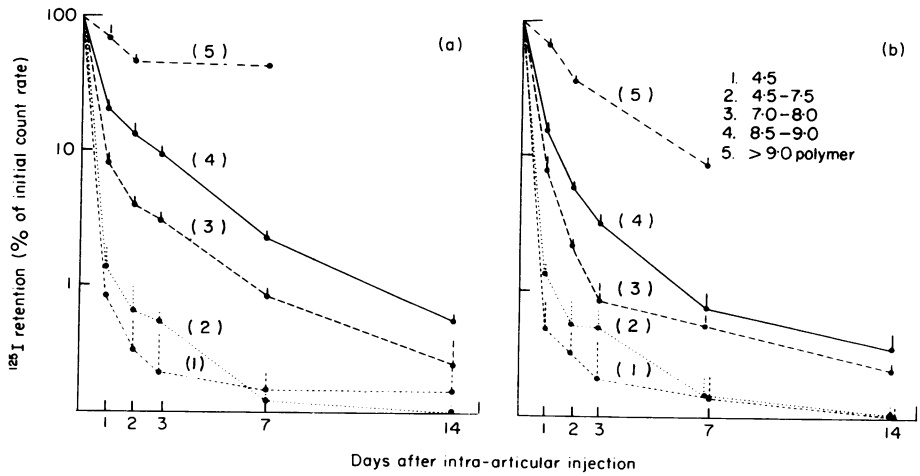


**Figure 2.** External radioactivity measurements at various days after intra-articular injection of 60 $\mu$g of monomeric $^{125}$I-native or modified BSA with various isoelectric points and highly cationic polymers in the joints of non-immune mice (a) and immune mice (b). Values represent the mean ± SD of groups of five mice.

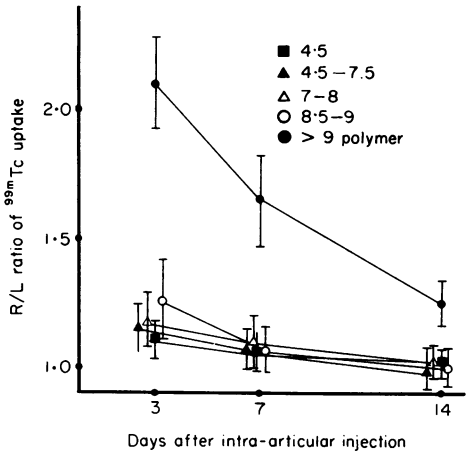


**Figure 3.** External radiation measurements 30 min after injection with 10 $\mu$Ci $^{99m}$Tc subcutaneously. Right/left ratios of the knee-joints > 1·10 were taken to indicate inflammation in the right knee-joint. Inflammation of knee-joints injected with monomer native and modified BSA and polymer cationic BSA at various days was determined.

### Arthritis induction

In order to investigate whether antigen retention correlates with chronicity, the severity of joint inflammation in immunized mice was measured with the $^{99m}$Tc uptake method and histological parameters. At several time-intervals (Days 3, 7 and 14) $^{99m}$Tc uptake in the right and left knee-joints was determined. Right/left ratios > 1 were taken to indicate inflammation of the right knee-joint.

Figure 3 shows that only cationic monomeric $BSA_{8\cdot5-9}$ is able to induce a severe chronic arthritis, whereas the monomeric less cationic derivatives but also the highly cationic polymer failed to induce arthritis. Two weeks after intra-articular injection, the animals were killed and the knee-joints were examined histologically. The arthritis induced with $BSA_{8\cdot5-9}$ was characterized by an abundant cellular exudate consisting predominantly of granulocytes. At Day 14 depletion of articular cartilage was seen as shown in Fig. 4b. In joints injected with the same dose of less cationic monomeric antigen (Fig. 4a) no gross histological changes were observed.

### Immunity to BSA derivatives

After the injection of the various BSA derivatives into knee-joints of non-immune mice, no inflammatory changes and no tissue destruction were seen. Therefore, the arthritis induced with $BSA_{8\cdot5-9}$ was highly dependent on the immune status of the animal. Apart from antigen retention, another determinant for the chronicity of arthritis could be that chemical modification of the antigen (by amidation) leads to an enhanced cellular immune response. We measured both the Arthus reaction and DTH. Four hours after the injection of antigen in the ear pinna of the immunized mouse the Arthus responses were similar for all antigens tested (Table 1). In the ELISA the titre of antibodies against BSA with higher pI (7–8, 8·5–9) was somewhat lower than against the BSA with lower pI (Table 1).

The state of cell-mediated immunity is shown by the increase in ear thickness at 24 hr. The DTH reaction was similar for all animals injected with the monomeric derivatives. Immunization with the polymeric BSA induced a good state of delayed-type hypersensitivity, which was evident when tested with $BSA_{8\cdot5-9}$. The polymeric BSA appeared to be a poor skin test antigen since it induced a granulomatous reaction and a high background in non-immune mice (Table 1).

### T-cell proliferation

Splenic T cells from mice primed with either form of the antigens were tested with the homologous antigen. The best response was found with $BSA_{7-8}$ (Fig. 5a). Native BSA only induced a good T-cell response when much higher antigen concentrations were used (data not shown). The enhanced response found with the cationic BSAs was not due to mitogenicity (Fig. 5f). In order to investigate which combination of immunization and antigen challenge would result in optimal T-cell response, cross experiments were performed (Fig. 5b–e). The best T-cell proliferation was found with $BSA_{7-8}$ regardless of the form of the antigen used for priming. The highly cationic polymeric form appeared to be toxic at concentrations over 2 $\mu$g per ml, and was not further used in these studies.

### Cross experiments

Since *in vitro* T-cell reactivity to the arthritogenic antigen $BSA_{8\cdot5-9}$ appeared to be higher upon priming with the less cationic BSA forms, *in vivo* cross experiments were performed. No consistent pattern of enhanced arthritis induction and chronicity was found after intra-articular injection of $BSA_{8\cdot5-9}$ in animals primed with $BSA_{7-8}$ and $BSA_{4\cdot5-7\cdot5}$.

## DISCUSSION

In this study we used a range of differently charged BSA species to determine the optimal arthritogenic antigen. Chronic murine antigen-induced arthritis could only be elicited with the cationic monomeric form $BSA_{8\cdot5-9}$. This antigen exhibited excellent retention in the joint combined with adequate T-cell reactivity.

In the model for antigen-induced arthritis, antigen is injected into the knee-joint of previously immunized animals. Earlier studies in the rabbit revealed that both sufficient antigen retention and delayed hypersensitivity are prerequisites for chronicity of the arthritis (Glynn, 1968). In the rabbit sufficient antigen retention in the joint could be achieved by antibody-mediated trapping (Cooke & Jasin, 1972). This mechanism appeared insufficient in mice, and enhanced retention could be achieved in this species using cationized antigens (van den Berg *et al.*, 1984, 1985). Charge-mediated retention of cationic BSA at the negatively charged joint structures was far superior to antibody-mediated trapping (van den Berg *et al.*, 1986).

In the present study various differently charged BSA species were tested for their arthritogenic properties in mice, and a correlation was sought with the degree of antigen retention and T-cell reactivity. Our retention studies made it clear that the higher the isoelectric point of the BSA species, the better the retention in the joint. However, if the degree of cationization is too high a polymeric form is obtained, and that species appeared to be a poor arthritogenic antigen. Therefore, excellent retention on its own seems to be insufficient. The monomeric derivative $BSA_{8\cdot5-9}$ exhibited excellent retention compared to native $BSA_{4\cdot5}$ and also a better T-cell reactivity, and this combination is probably responsible for the observed arthritogenicity. The less

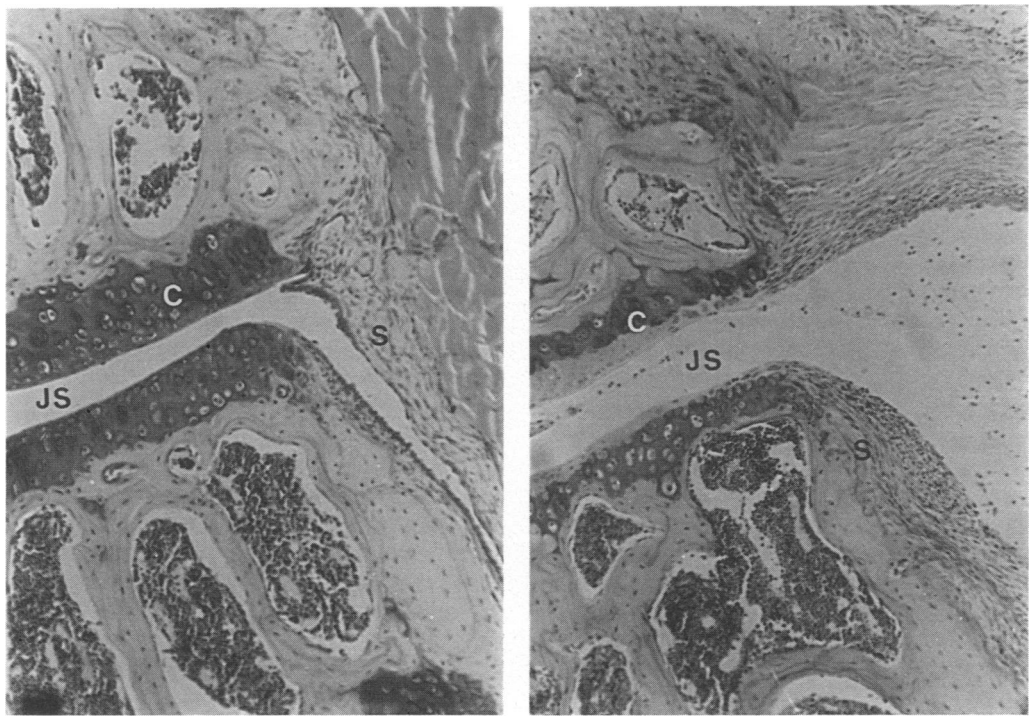


**Figure 4.** Knee-joint of previously immunized mice 14 days after intra-articular injection with BSA pI 7–8 (a) and BSA pI 8·5–9 (b). BSA pI 8·5–9 induces arthritis with depletion of cartilage, exudate in the joint cavity and infiltration of inflammatory cells in the synovium. In the joint injected with BSA pI 7–8 no changes are seen. (Safranin O, magnification ×80; C: cartilage, S: synovium, JS: joint space.

**Table 1.** Immune status after immunization

| Group | Immunization (pI BSA) | Skin test (pI BSA) | Ear test 4 hr | Ear test 24 hr | ELISA Day −1 | ELISA Day 15 |
|---|---|---|---|---|---|---|
| 1 | 4·5 | 4·5 | 24±2 | 24±3 | 12±1 | 13±1 |
| 2 | 4·5–7 | 4·5–7 | 19±2 | 23±4 | 12±1 | 13±1 |
| 3 | 7–8 | 7–8 | 23±4 | 24±3 | 10±1 | 10±1 |
| 4 | 8·5–9 | 8·5–9 | 22±2 | 26±5 | 11±1 | 11±1 |
| 5 | >9·5 | 8·5–9 | 19±6 | 26±7 | — | — |
| 6 | >9·5 | >9·5 | 24±4 | 10±4 | — | — |
| 7 | — | 8·5–9 | 10±1 | 7±3 | — | — |
| 8 | — | >9·5 | 17±6 | 23±4 | — | — |

Data of ELISA are the mean of the determinations in sera of at least five mice.

* Mean ± SD, expressed as two log values, using 50% of the maximal extension as end-point. Data of DTH are the mean values ± SD of increase in ear thickness (mm × $10^{-2}$) corrected for the control values in non-immune mice, measured 4 hr and 24 hr after the injection of 5 μg antigen in 10 μl saline into the pinna of the ear.

cationic form $BSA_{7-8}$ showed approximately three times less antigen retention compared to $BSA_{8\cdot5-9}$, but apparently is an even better T-cell antigen. Although the DTH reactions were similar, $BSA_{7-8}$ was a better priming antigen and the best challenge antigen in the T-cell proliferation assays. Nevertheless, it failed to induce sustained arthritis. This would suggest that, given a basal level of T-cell reactivity, antigen retention is the more important denominator for chronicity of arthritis.

Additionally, other characteristics of $BSA_{8\cdot5-9}$ may be important determinants for the induction of chronic inflammation. Intra-articular injection of 60 μg $BSA_{8\cdot5-9}$ causes some aspecific acute inflammation (unpublished obvservations), which is probably related to its high cationicity. This event may be a relevant extra trigger to promote influx of antigen-reactive cells, leading to a vigorous allergic reaction. Moreover, $BSA_{8\cdot5-9}$ shows a high affinity for cartilage. Above isoelectric point 8·5–9, a sharp increase was found in protein retention in hyaline cartilage structure, and such proteins deeply penetrate the dense cartilage matrix (van den Berg *et al.*, 1985; Schalkwijk *et al.*, 1985; van Lent *et al.*, 1987). This indicates that, apart from quantitative differences, qualitative differences also exist between the retention (in joint structures) of $BSA_{8\cdot5-9}$ compared with $BSA_{7-8}$. Perhaps the presence of a reservoir of retained antigen in the cartilage, providing sustained leakage of small amounts of antigen in the joint cavity, plays an important role in perpetuation of arthritis.

The observation that a three times lower dose of highly cationic BSA (20 μg) causes less severe but sustained inflammation provides further evidence for the notion that the difference in arthritogenicity between $BSA_{8\cdot5-9}$ and $BSA_{7-8}$ is not simply due to the three times difference in antigen retention. The observation that the highly cationized polymeric BSA form does not induce arthritis needs further discussion. One explanation could be that, due to a high degree of aggregation, relatively few antigenic determinants are exposed. Another important fact is the high toxicity of the preparation. This was found in our *in vitro* T-cell proliferation, and similar toxicity findings with highly cationized BSA were recently reported (Muckerheide *et al.*, 1987). Further evidence along this line comes from transfer studies. T-cell clones directed against cationic methylated BSA

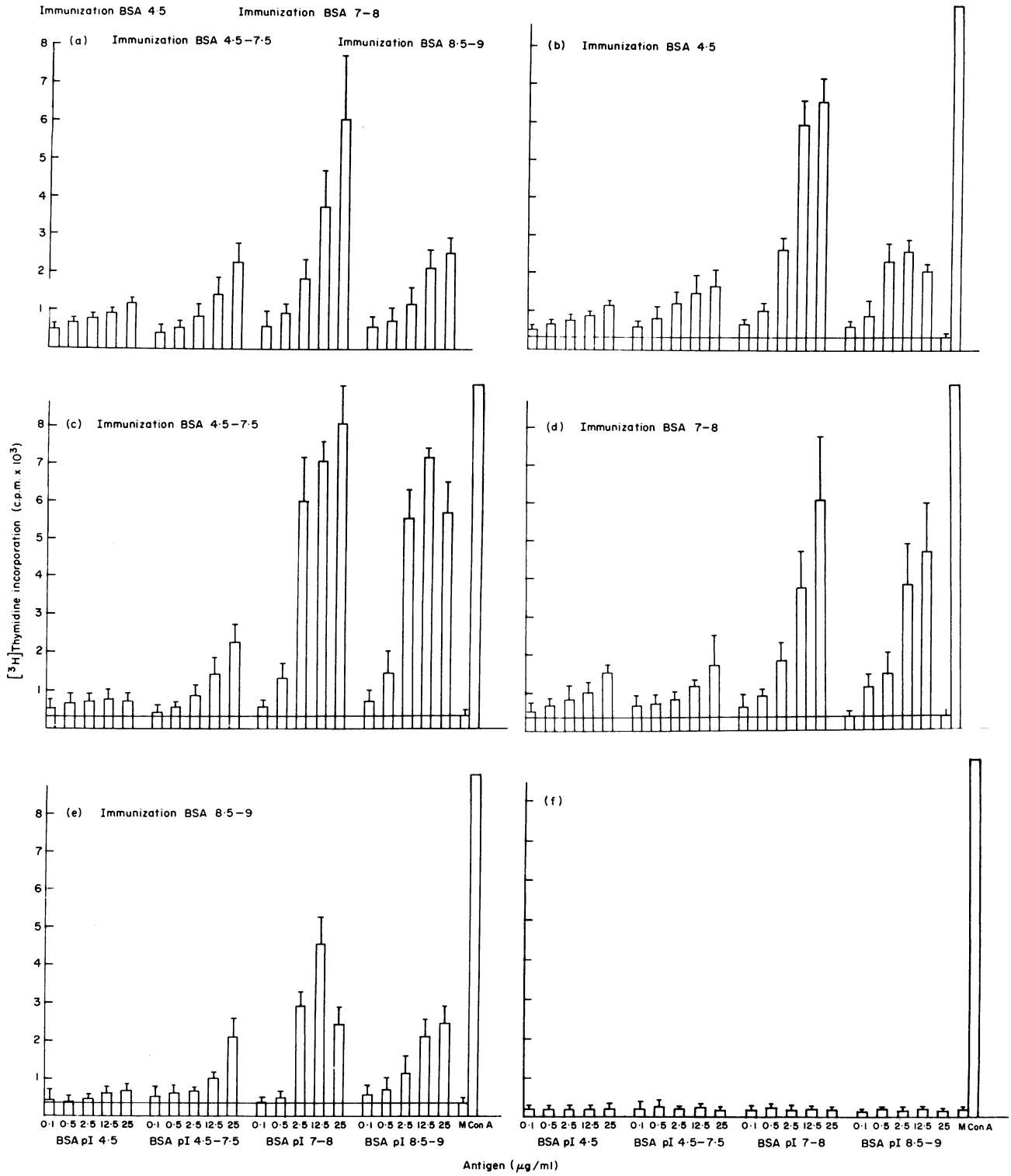


**Figure 5.** [$^3$H]Thymidine incorporation in c.p.m. of spleen T lymphocytes derived from mice immunized with BSA of different isoelectric points and stimulated with the antigen with which they were immunized (a) and spleen lymphocytes derived from immunization with BSA pI 4·5 (b), pI 4·5–7·4 (c), pI 7–8 (d), pI 8·5–9 (e) and non-immunized (f), cultured for 4 days in the presence of various concentrations (0·1, 0·5, 2·5, 12·5 and 25 μg/ml) with native and chemically modified BSA with different isoelectric points.

(mBSA) failed to induce arthritis when injected together with the toxic antigen, whereas separate injections gave optimal results (I. S. Klasen, R. M. T. Ladestein, I. G. Donselaar, W. B. van den Berg, R. Tees and R. Benner, manuscript submitted for publication). Although apparently not suitable for induction of inflammation, the polymeric form can be used for priming. It induces a good T-cell immunity against the less cationic derivatives.

There are clear indications to suggest that chemical modification of a protein changes its immunological properties. For instance, upon immunization with native lysozyme a poor T-cell response developed, and this could be highly enhanced using carboxymethylated lysozyme. (Allen & Unanue, 1984). This was also found with lipid-conjugated BSA (Dailey & Hunter, 1977). Methylated BSA caused a shift towards T-cell immunity (Crowle, Hu & Patrucco, 1968). The reason for a better T-cell reaction against cationized proteins may be related to the interaction with antigen-presenting cells. Cationic proteins show a high affinity for anionic cell membranes and therefore tend to adhere better (Renau-Piqueras, Miragall & Cervera, 1985). Moreover, the processing of such proteins seems different. Cationic proteins partly escape from destruction in the lysosomal compartment (Stenseth, Hedin & Thyberg, 1983). Importantly, the sticking of cationized proteins to cell membranes does not necessarily make them mitogenic. In our hands neither methylated nor amidated BSA showed mitogenic effects in T-cell proliferation assays.

Another interesting point is the specificity of the T-cell reaction against the introduced polycationic groups. Arthritis and T-cell reactivity can be induced with amidated ovalbumin (aOA) and aBSA (van den Berg *et al.*, 1987), but no cross-reactivity was observed. A similar lack of cross-reactivity at the T-cell level was described for methylated proteins (Lens *et al.*, 1983). It seems likely that some cross-reactivity should develop when different proteins are cationized to a very high degree. $BSA_{8\cdot5\text{-}9}$ probably contains both new determinants, related to cationization, and old determinants of the native molecule, and the T-cell reaction upon priming is directed against both. $BSA_{8\cdot5\text{-}9}$ does induce short-lasting but clearly allergic inflammation in native BSA-primed mice (data not shown). The fact that $BSA_{8\cdot5\text{-}9}$ does induce chronic inflammation in BSA-primed animals suggests that ongoing inflammation relates to continued triggering of T cells reactive against determinants introduced by cationization. As discussed in the previous paragraph, this interaction probably needs quite a lot less antigen compared with native BSA due to excellent presentation. This may have relevance for chronicity since tiny amounts of antigen are slowly released from the cartilage reservoirs over long periods in this model of arthritis.

About 20% of the naturally occurring proteins possess a pI above 8·5 (Gianazza & Righetti, 1980). Some bacterial species such as the nephritogenic *Streptococcus maracaibo* excrete extracellular products with a pI of 8·5–9. These proteins accumulate in the kidney glomeruli if injected intravenously, and are thought to play a role in post-streptococcal nephritis (Vogt *et al.*, 1983). Besides showing an excellent retention, these proteins are potent T-cell stimulators. In very low concentrations they are able to stimulate T cells from patients with post-streptococcal nephritis (Susuki & Vogt, 1986). The behaviour of naturally occurring cationic proteins in relation to joint structures and their possible potency to induce an inflammatory response are further areas for investigation.

## ACKNOWLEDGMENTS

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## REFERENCES

ALLEN P.M. & UNANUE E.R. (1984) Differential requirements for antigen processing by macrophages for lysozyme-specific T cell hybridomas. *J. Immunol.* **132,** 1077.

VAN DEN BERG W.B., JOOSTEN L.A.B., VAN DE PUTTE L.B.A. & ZWARTS W.A. (1987) Electric charge and joint inflammation: suppression of cationic aBSA induced arthritis with a competitive polycation. *Am. J. Pathol.* **127,** 15.

VAN DEN BERG W.B., KRUIJSEN M.W.M. & VAN DE PUTTE L.B.A. (1982) The mouse patella assay. An easy method of quantitating articular cartilage function *in vivo* and *in vitro*. *Rheumatol. Int.* **1,** 165.

VAN DEN BERG W.B., VAN LENT P.L.E.M., VAN DE PUTTE L.B.A. & ZWARTZ W.A. (1986) Electrical charge of hyaline articular cartilage: its role in the retention of anionic and cationic proteins. *Clin. Immunopath.* **39,** 187.

VAN DEN BERG W.B. & VAN DE PUTTE L.B.A. (1985) Electrical charge of the antigen determines its localization in the mouse knee joint. Deep penetration of cationic BSA in hyaline articular cartilage. *Am. J. Pathol.* **121,** 224.

VAN DEN BERG W.B., VAN DE PUTTE L.B.A., ZWARTS W.A. & JOOSTEN L.A.B. (1984) Electrical charge of the antigen determines intraarticular antigen handling and chronicity in mice. *J. clin. Invest.* **74,** 1850.

BRACKERTZ D., MITCHELL G.F. & MACKAY I.R. (1977) Antigen induced arthritis in mice. I. Induction of arthritis in various strains of mice. *Arthritis Rheum.* **20,** 841.

CONSDEN R., DOBLE A., GLYNN L.E. & NIND A.P. (1971) Production of a chronic arthritis with ovalbumin. Its retention in the rabbit kneejoint. *Ann. Rheum. Dis.* **30,** 107.

COOKE T.D. & JASIN H.E. (1972) The pathogenesis of chronic inflammation in experimental antigen induced arthritis. I. The role of antigen on the local immune response. *Arthritis Rheum.* **15,** 327.

CROWLE A.J., HU C.C. & PATRUCCO A. (1968) Preferential development by mice of delayed hypersensitivity to purified basic proteins. *J. Allergy*, **42,** 140.

DAILEY M.O. & HUNTER R.L. (1977) Induction of cell-mediated immunity to chemically modified antigens in guinea pigs. I. Characterisation of the immune response to lipid-conjugated protein antigens. *J. Immunol.* **118,** 957.

DANON D., GOLDSTEIN L., MARIKOVSKY Y. & SKUTELSKY E. (1972) Use of cationized ferritin as a label of negative charges on cell surfaces. *J.Ultrastruct. Res.* **38,** 500.

GIANAZZA E. & RIGHETTI P.G. (1980) Size and charge distribution of macromolecules in living systems. *J. Chromatogr.* **193,** 1.

GLYNN L.E. (1968) The chronicity of inflammation and its significance in rheumatoid arthritis. *Ann. Rheum. Dis.* **27,** 105.

HUNTER W.M. & GREENWOOD F.C. (1962) Preparation of $^{131}$I-labelled growth hormone of high specific activity. *Nature (Lond.)*, **194,** 495.

LAEMMLI U.K. (1970) Cleavage of structural proteins during the assembly of bacteriophage T4. *Nature (Lond.)*, **227,** 680.

LENS J.W., VAN DEN BERG W.B. & VAN DE PUTTE L.B.A. (1983) Flare-up of antigen-induced arthritis in mice after challenge with intravenous antigen: studies on the characteristics of and mechanisms involved in the reaction. *Clin. exp. Immunol.* **55,** 287.

VAN LENT P.L.E.M., VAN DEN BERG W.B., SCHALKWIJK J., VAN DE PUTTE

L.B.A. & VAN DEN BERSSELAAR L. (1987) The impact of protein size and charge on its retention in articular cartilage, *J. Rheum.* (in press).

MUCKERHEIDE A., APPLE R.J., PESCE A.J. & MICHAEL J.G. (1987) Cationization of protein antigens. I. Alteration of immunogenic properties. *J. Immunol.* **138,** 833.

RENAU-PIQUERAS J., MIRAGALL F. & CERVERA J. (1985) Endocytosis of cationized ferritin in human resting peripheral blood by T lymphocytes. *Cell Tiss. Res.* **240,** 743.

SCHALWIJK J., VAN DEN BERG W.B., VAN DE PUTTE L.B.A., JOOSTEN L.A.B. & VAN DEN BERSSELAAR L. (1985) Cationization of catalase, peroxidase and superoxidedismutase. Effect of improved intra-articular retention on experimental arthritis in mice. *J. clin. Invest.* **76,** 435.

STENSETH K., HEDIN U. & THYBERG J. (1983) Endocytosis, intracellular transport, and turnover of anionic and cationic proteins in cultured mouse peritoneal macrophages. *Eur. J. Cell Biol.* **31,** 15.

SUZUKI M. & VOGT A. (1986) Mitogenic activity of extracellular cationic products produced by group A streptococci: analysis of the lymphocyte response. *Clin. exp. Immunol.* **66,** 132.

VOGT A., BATSFORD S., RODRIGUEZ-ITURBE B. & GRACIA R. (1983) Cationic antigens in poststeptococcal glomerulonephritis. *Clin. Nephrol.* **20,** 271.