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Allergic arthritis induced by cationic proteins: role of molecular weight

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SUMMARY

Previous studies have shown that chronic murine allergic arthritis can only be induced with cationized BSA, related to excellent retention of the cationic antigen in the joint. We now investigate the impact of size of cationic proteins on their potential to induce this form of arthritis. After intra-articular injection, antigen retention is much enhanced with high molecular weight cationized proteins, like albumin or immunoglobulin, compared to small-sized proteins like myoglobulin and lysozyme. Consequently, severe chronic arthritis was only found with the former ones. The role of size is further substantiated with poly-L-lysine-coupled lysozyme. This derivative shows excellent retention *in vivo* and causes a chronic destructive arthritis in preimmunized mice, in contrast to the poor arthritis seen with native cationic lysozyme. Control experiments made it clear that antigen retention is the most important denominator and that differences in chronicity are not related to gross variations in T-cell reactivity. Retention studies *in vitro* revealed that the potential to bind to joint structures is similar for the various proteins, suggesting that *in vivo* conditions determine size-related differences in antigen clearance. Our data indicate that cationicity *per se* does not make a protein a proper arthritogen.

INTRODUCTION

It has been shown recently that cationic antigens are potent triggers for the induction of chronic allergic arthritis in mice (Van den Berg *et al.*, 1984). Highly cationic bovine serum albumin (BSA), injected directly into the joint of preimmunized mice, resulted in severe long-lasting arthritis, characterized by prolonged influx of granulocytes in the joint cavity and synovial tissue. The chronicity of the arthritis is eventually responsible for severe cartilage destruction (Van den Berg *et al.*, 1981), and abnormalities found closely resemble lesions observed during rheumatoid arthritis. In contrast to highly cationic BSA, the native anionic (Van den Berg *et al.*, 1984) but also less cationic derivatives (Van Lent *et al.*, 1987) of BSA failed to induce chronic inflammation. It is generally accepted that in the model of antigen-induced arthritis two prerequisites are of eminent importance: sufficient retention of antigen in the joint structures and adequate T-cell reactivity against the retained antigen (Brackertz, Mitchell & Mackay, 1977; Van den Berg *et al.*, 1984).

To define further essential properties of potential arthritogenic cationic proteins we recently determined both antigen retention, T-cell reactivity and arthritis induction for a range of differently charged BSA derivatives. It turned out that the isoelectric point (IEP) of these proteins needs to be above 8·5 to obtain strong charge-mediated retention in joint structures, both *in vitro* and *in vivo* (Van Lent *et al.*, 1987), and that these derivatives are also better T-cell antigens compared to native anionic BSA (Van Lent *et al.*, 1987; Apple *et al.*, 1988). This combination makes such proteins highly arthritogenic.

When overviewing the spectrum of naturally occurring cationic proteins it became evident that about 20% of natural proteins have an IEP above 8·5, but that most of those proteins have a molecular weight (MW) below 30,000 (Gianazza & Righetti, 1980). The objective of the present study was to delineate whether size is an important determinant for arthritogenic potential. We chose a range of proteins with increasing MW for 12,000 to 150,000 that were cationized by optimal amidation to obtain cationic derivatives of equal charge. The small-sized naturally occurring cationic protein lysozyme was tested both in its native form and as a high molecular derivative after complexing with poly-L-lysine. Our data indicate that size is indeed an important factor determining antigen retention in joint tissues *in vivo*, and that sufficient cationicity *per se* does not guarantee arthritogenic potential in the model of murine antigen-induced arthritis.

MATERIALS AND METHODS

Animals

Male C57B1 mice, aged 8–10 weeks at the start of the immunization, were used.

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Materials

Myoglobulin horse (Myo), B lactoglobulin (Lact) from bovine milk (three times crystalysed and lyophylized), chicken serum albumin (OA), bovine serum albumin (BSA) (fraction V), human immunoglobulin (IgG), hen's egg lysozyme (Lys) 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. The 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, 400 μl of DMPA in 20 ml PBS were adjusted with 1N HCL to pH 5·0. In addition 400 mg protein and 300 mg of EDC were added. After stirring for 4 hr the solution was dialysed against distilled water and the modified proteins were freeze-dried. In this way free carboxyl groups of the protein are coupled to aminogroups of DMPA. The IEP of the protein will thus be raised, since anionic groups are eliminated and cationic groups are introduced by DMPA.

Lysozyme was coupled to poly-L-lysine using EDC as activator. The coupled poly-L-lysine was separated from free lysozyme by Sephadex G50 gel filtration. The IEP of the coupled Lys varied from 8·5 to 9·5, as determined by isoelectrofocusing. The ratio lysozyme/poly-L-lysine in the coupled product was determined according to the method of Shen, Yang & Ryser (1984) and was found to be 1.

Protein characterization

The isoelectric point of the various proteins was determined in a 5% polyacrylamide slab gel with 0·8% ampholines (pH gradient from 3·5 to 9·5) (isoelectrofocussing gels).

In addition, further characterization of the cationicity of the proteins was done with the method of Habeeb (1966). In this assay the substitution of ethylenediamine onto protein is determined by photospectrometric analysis for aminogroups using trinitrobenzenesulphonic acid (TNBS). SDS–PAGE was performed according to Laemli (1970). Proteins were separated on a 7–18% gradient polyacrylamide slabgel 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. The labelled derivatives were free of low molecular weight degradation products.

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 (2×10^9) *Bordetella pertussis* organisms (National Institute of Public Health, Bilthoven, The Netherlands) were administered intraperitoneally as an additional adjuvant. Booster injections were given subcutaneously in the neck region on Day 7.

Skin testing

Arthus reaction and delayed hypersensitivity were measured at 6, 24, 48 and 72 hr, respectively, after injection of 5 μg antigen in 10 μl saline into the pinna of the ear. Increase in ear thickness was measured with an engineers micrometer.

Antigen-retention measurements

^{125}I-labelled antigen (60 μg) was injected in the right knee joint wherease the left knee joint received saline. At various days thereafter (see results) ^{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, 14 groups of mice were anaesthesized by ether inhalation.

^{99m}Tc uptake measurements

Joint inflammation was determined by ^{99m}Tc pertechnetate uptake measurements of the kneejoint. This method has been shown to correlate well with histological findings (Lens, Van den Berg & Van de Putte, 1984). Briefly mice were injected intraperitoneally with 12 μCi ^{99m}Tc and sedated with chloralhydrate. After 30 min the amount of radioactivity was assessed by measuring the gamma radiation with the knee in a fixed position, using a collimated Na-I-scintillation crystal. Arthritis was scored as the ratio of the ^{99m}Tc uptake in the right (R) and the left (L) kneejoint. R:L ratios $> 1:1$ were taken to indicate inflammation of the right knee.

Antigen retention in vitro

Patellae with surrounding tissue were removed from the knee joint of normal C57B1 mice as described previously (Van den Berg *et al.*, 1982). The patellae with adjacent tissue were incubated in microtitre plates in 100 μl PBS 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 ng per patella or surrounding tissue.

For the elution studies, patella specimens were preloaded with radiolabelled protein for 2 hr and additionally post-treated with 100 μl (10 mg/ml) and 0·5 M NaCl during 2 hr at 37° and subsequently processed as described above.

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 of the sera followed by detection of bound mouse Ig with 1:400 diluted peroxidase-conjugated rabbit anti-mouse Ig (Miles Laboratories Inc., Elkhart, IN). O-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 endpoint.

Table 1. Charge characterization of cationic(c) proteins

Protein	MW	I.E.F. gel	No. free amino groups per 10,000 MW
cMyo	18	9–9·5	12·6
cLact	30	9–9·5	10·6
cOA	47	9–9·5	7
cBSA	67	9–9·5	11·3
cIgG	150	9–9·5	10·
Lys	14	11*	5

Differently sized proteins, modified by optimal amidation and native cationic lysozyme, were characterized on I.E.F. gels and by determination of the number of free amino groups per surface- unit of the protein.

*According to the literature (Righetti & Caravaggio, 1976).

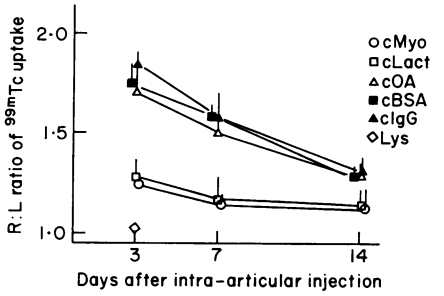

Figure 1. Right:left ratio of ^{99m}Tc uptake at various days after intra-articular injection of cationic antigens of different size into the right knee joint. R:L ratios of the knee joints >1:1 were taken to indicate inflammation. Data represent the mean ± SD of five mice.

RESULTS

Protein characterization

The charge of the native proteins was changed by optimal amidation. This resulted in cationic proteins with IEP between 9 and 9·5. In addition, we determined the number of free amino groups on the outer surface of the protein (Table 1). If corrected for the molecular weight, all proteins have quite similar amounts of free amino groups, except for native lysozyme which has relatively few.

Arthritis induction

After intra-articular injection of the proteins in the knee joint of preimmunized animals, the severity of the joint inflammation was measured by ^{99m}Tc uptake and histology. All high molecular weight proteins [cationic(c) IgG, cBSA and cOA] show severe inflammation, whereas the smaller proteins cMyo and cLact induced a mild arthritis (Fig. 1). These differences were confirmed by histology at Day 14 and Day 21 (Fig. 2). In contrast, lysozyme only induced very short-lasting joint inflammation, which was not even detectable anymore at Day 3. Since it is known from previous work with various forms of BSA that chronicity is related to both antigen retention in the joint and adequate delayed hypersensitivity against the retained antigen, those two parameters were investigated (Van Lent *et al.*, 1987).

Immunity to cationic proteins

The severity of arthritis is highly dependent on the immune status of the animal. A major determinant for chronicity of arthritis is a good cellular response. We both measured Arthus and DTH reaction by increased ear-thickness several time-points after injection of 5 μg antigen in the pinna of the right ear.

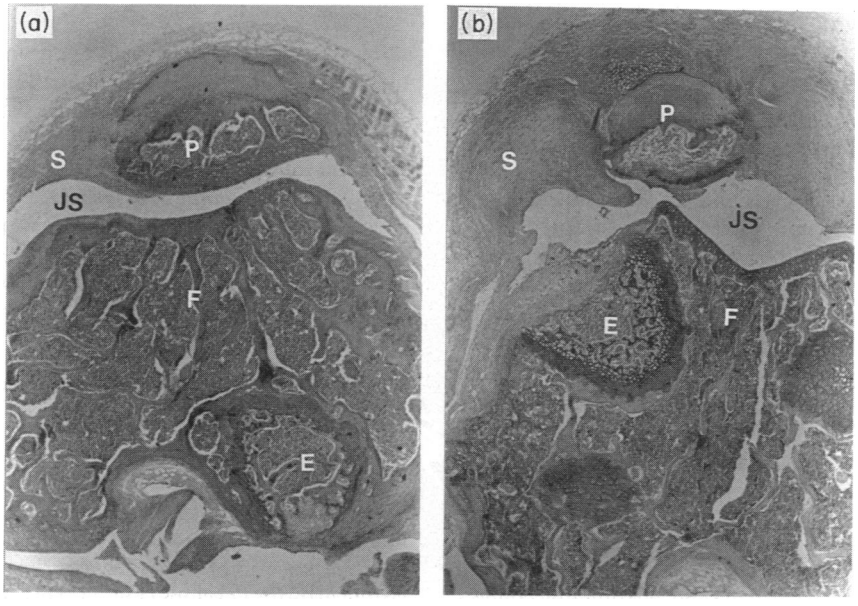

Figure 2. Total knee joint section of previously immunized mice, 14 days after intra-articular injection with (a) cMyo (MW. 18,000, pI 9–9·5) and (b) cIgG (MW 150,000, pI 9–9·5). cIgG induces more severe arthritis, characterized by elevated amounts of inflammatory cells in the synovium (safranin o, magnification ×28. P, patella; S, synovium; F, femur; JS, joint space. E, epiphysial plates.)

Table 2. Immune status after immunization

Group	Immunization	Skin test	Ear test (hr) 6	24	48	72	ELISA Day−1
1	cMyo	cMyo	17±2	19±2	19±1	20±2	12±1
2	cLact	cLact	17±1	18±2	20±4	21±3	11±1
3	cOA	cOA	22±2	24±1	28±6	27±3	12±1
4	cBSA	cBSA	16±2	18±1	19±3	19±2	11±1
5	cIgG	cIgG	19±1	20±3	22±1	21±2	13±1
6	Lys	Lys	17±1	17±2	12±1	11±2	10±2
7	Lys	PLL-Lys	19±2	20±3	22±4	23±1	10±2

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 extinction as the end-point.

Data of DTH are the mean values ± SD of increase in ear-thickness, ($mm \times 10^{-2}$) corrected for the control values in non-immune mice, measured 6, 24, 48 and 72 hr after injection of 5 μg antigen in 10 μl saline into the pinna of the ear. Control values never exceeded 10 ± 3 at 24 hr with the exception for PLL-Lys which was 15 ± 4 at this time-point.

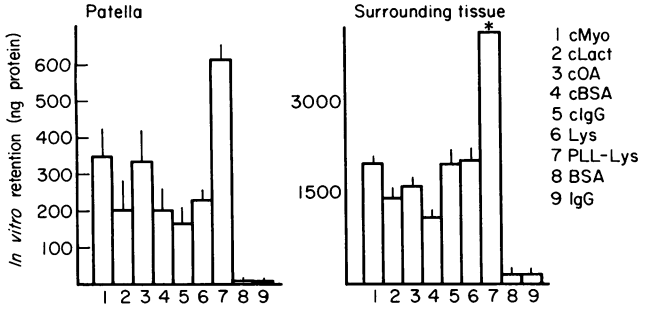

Figure 3. *In vitro* retention of cationic proteins to joint tissue. Patellae specimens were incubated with ^{125}I-labelled proteins at concentrations of 0·3 mg/ml for 2 hrs at 37°. Values represent the mean ± SD of groups of five patellae; *2574 ± 466 ng.

Six hours after intra-auricular injection of the antigen, corresponding Arthus responses were found for the antigens tested (Table 2). Antibody responses directed against the cationic proteins as determined by ELISA were also in the same order of magnitude. After 24 hr all proteins also induced significant DTH reactions which were sustained after 48 and 72 hr, indicating that these values did not represent remnants of the Arthus reaction (Table 2). As expected cOA appeared to be a strong T-cell antigen. Native lysozyme showed a good response at 24 hr but a sharp decline by 48 hr.

Antigen retention

Retention of the various proteins was studied both *in vitro* and *in vivo*, using ^{125}I-labelled derivatives. All cationic proteins showed excellent retention *in vitro* in cartilage and surrounding connective tissue, irrespective of molecular weight (Fig. 3). For comparison, we included the minimal retention found for a small and a large anionic protein. In contrast to the situation *in vitro*., the retention *in vivo* upon intra-articular injection appeared to be highly dependent on the molecular weight of the cationic proteins. Large proteins persisted significantly better than smaller ones (Fig. 4), and this was found both in immune and non-immune animals. A striking observation was the extremely rapid clearance of lysozyme from the joint. Although having a similar pI as the cationized proteins and having a comparable potential to bind to joint structures *in vitro*, the retention *in vivo* was nevertheless very poor. This easily explains the failure of lysozyme to induce sustained arthritis.

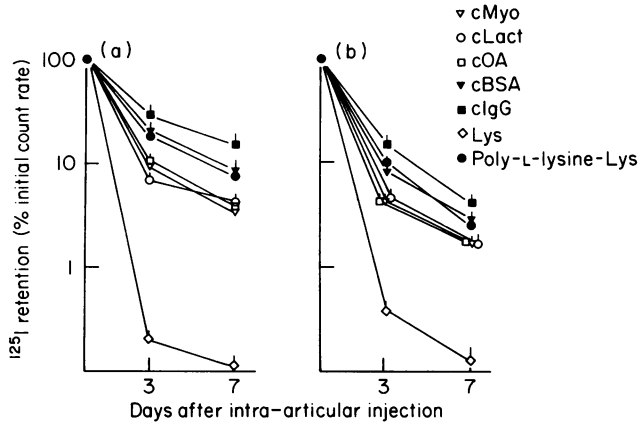

Figure 4. External radioactivity measurements at various days after intra-articular injection of 60 μg of ^{125}I-labelled native or modified (amidation; PLL coupling) cationic proteins in the joints of non-immune mice (a) and immune mice (b). Values represent the mean ± SD of groups of five mice.

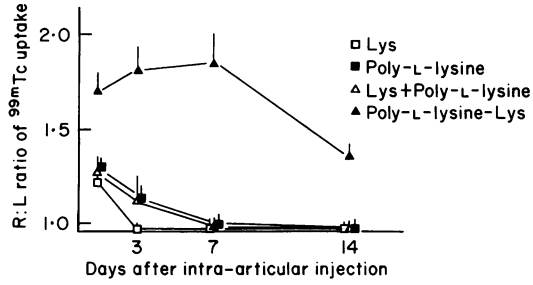

Figure 5. R:L ratios of ^{99m}Tc uptake at various days after intra-articular injection of 60 μg of native and PLL-coupled lysozyme. PLL alone or in combination with lysozyme (not coupled) were also tested. Values represent the mean ± SD of five mice.

Arthritis induction with poly-L-lysine-lysozyme

To further substantiate the importance of antigen retention for arthritis induction, we coupled lysozyme to the cationic polymer poly-L-lysine (PLL). PLL-Lys showed again excellent retention *in vivo* (Fig. 4), to the order of magnitude expected on account of its molecular weight (70,000). When this derivative is injected into the knee joint of lysozyme-primed mice a severe, long-lasting arthritis develops (Fig. 5). This chronicity was related to the increase in persistence of lysozyme and not to the mere presence of PLL, since PLL alone or in combination with lysozyme (not coupled) did not induce chronic inflammation.

Table 3. Binding strength of cationic proteins in joint tissue

	Amount of protein retained (%)			
	Patellar cartilage		Surrounding tissue	
Post-treatment	Lys	cMyo	Lys	cMyo
(a) 0·15 M NaCl	100	100	100	100
Protamine 10 mg/ml	13±2*	17±3	27±5	64±3
0·5 M NaCl	14±3	13±1	32±3	71±4
(b) 0·15 M NaCl	100	100	100	100
Protamine 10 mg/ml	7±1	22±2	42±6	65±4
0·5 M NaCl	9±2	22±3	44±4	62±3

Patella specimen were incubated with ^{125}I-labelled Lys and cMyo at concentrations of 0·3 and 3 mg/ml, followed by a 2-hr incubation (post-treatment) with ionic binding-impairing agents, protamine (10 mg/ml) or 0·5 M NaCl.

*Values represent the mean ±SD of five tissue specimens.

Character of binding of cationic proteins in joint tissue

To explore whether the binding strength of lysozyme to joint tissue was different compared to that of cationized proteins, patella specimens were first incubated with ^{125}I-labelled lysozyme or cationized myoglobulin, which have comparable size. Subsequently, post-treatment with protamine or 0·5 M NaCl revealed that lysozyme could be more effectively eluted from surrounding tissue than amidated myoglobulin (Table 3), indicating that chemically modified proteins bind stronger to negatively charged joint tissue.

DISCUSSION

Small and large sized cationized proteins, all proper T-cell antigens, appeared to be arthritogenic upon administration in joints of previously immunized mice. However, large cationic proteins (cIgG, cBSA) caused significantly more chronic inflammation and tissue damage compared to smaller sized molecules (cMyo, Lys), which was clearly related to antigen retention.

Previous studies revealed that antigen trapping in joint tissues for extended periods elicits chronic arthritis in preimmunized animals (Consden *et al.*, 1971; Cooke & Jasin, 1972; Van den Berg *et al.*, 1984). This trapping may be mediated by antibodies (Hollister & Mannik, 1974; Cooke, Hurt & Ziff, 1972; Jasin, 1975; Teuscher & Donaldson, 1979; Aston, Ward & Cooke, 1983) but far more effectively by charge-mediated mechanisms (Van den Berg *et al.*, 1984, 1986). Recently it was demonstrated that cationic BSA with a pI larger than 8·5 provokes severe chronic arthritis after injection in the knee joint of preimmunized mice, whereas the native anionic (Van den Berg *et al.*, 1984) or less cationic forms (Van Lent *et al.*, 1987) failed in this respect. The severity of arthritis was clearly related to the amount of persisting antigen but also to T-cell immunity (Van den Berg *et al.*, 1984; Van Lent *et al.*, 1987). Our finding that smaller, charge-modified cationic proteins cause milder arthritis than larger molecules was not due to differences in cellular or humoral immunity. Both DTH, as measured by increase in ear thickness, and antibody titre, as measured by ELISA, were of the same order of magnitude. However, *in vivo* retention studies in knee joints of preimmunized mice showed reduced binding of the small compared to large sized proteins, although having corresponding charge, as expressed on isoelectrofocusing gells and number of free amino groups per surface unit. In contrast, *in vitro* binding of small and large cationic proteins was similar. This indicates that under *in vivo* conditions other mechanisms are involved which may impair initial binding to or elution of already bound proteins from negatively charged joint tissue. Size of cationic proteins seems to be an important denominator for *in vivo* binding. Clearance of anionic proteins from the joint cavity via the lymphatics do not seem to be size dependent (Wallis *et al.*, 1985).

In contrast to the modified proteins, native cationic lysozyme only elicits a very short-lasting arthritis. At Day 3 no signs of inflammation were detected any more. This lack of arthritogenicity is not related to poor T-cell immunity against this protein. Although DTH was low after 48 hr, Lys appeared to be a good T-cell antigen in the *in vitro* T-cell proliferation assay (data not shown). The low DTH may be explained by the fast removal of lysozyme from the ear, as suggested by signs of shock, shortly after injection of the protein. The failure of lysozyme to induce severe arthritis may well be explained by the poor binding to joint structures *in vivo*. Despite the high isoelectric point only minimal amounts were detected 1 day after injection. That minimal binding was indeed the reason for the very mild arthritis was proved by PLL-coupled lysozyme. The 70,000 MW complex, in which the PLL:Lys ratio was 1, persisted in much larger amounts to the joint compared to natural lysozyme, and elicited a very severe chronic arthritis. PLL alone or PLL together with lysozyme (but in free form) were not effective. Although lysozyme and cationic myoglobulin are of corresponding size and charge, the last one did elicit at least a moderate arthritis, in line with much enhanced antigen retention in the joint compared with lysozyme. Further characterization of these cationic molecules by free amino group determination revealed that cationic myoglobulin exposed twice as much amino groups on its outer surface compared with lysozyme. Elevated exposure of free amino groups on the protein surface may well be responsible for stronger electrostatic binding. This phenomenon was, however, again only seen *in vivo*. *In vitro* retention studies demonstrated a comparable degree of persistence. This suggests that under *in vivo* conditions, binding of proteins like lysozyme is less effective.

To obtain more evidence about the potential variation in the strength of binding of proteins with different number of free amino groups, patella specimens were preloaded with Lys or cationic myoglobulin, followed by elution with ionic binding impairing agents, protamine and 0·5 M NaCL. Protamine, a highly cationic protein may compete for anionic sites (Van den Berg *et al.*, 1987), in this way dissipating cationic proteins already present, whereas under high salt condition ionic interaction is diminished. It was found that with both treatments, Lys eluted more effectively from joint tissue than cMyo, indicating weaker binding of lysozyme with joint matrix components.

This study provides evidence that cationic proteins may be potent triggers for sustained arthritis but that cationicity *per se* does not make a protein a proper arthritogen. Size and density, and perhaps also the type of positively charged groups on the outer surface of the protein, may be important properties for prolonged binding and so for arthritogenicity. Localization of

charged antigens and its implication for inflammation has also been studied in the kidney. Significant glomerular binding occurred when the isoelectric point exceeded a threshold value of 8·5–9·5, whereas at a given high isoelectric point antigen binding increased with molecular weight (Vogt *et al.*, 1982). Acting as a target for specific antibody molecules, the *in situ*-formed immune complexes may direct glomerulonephritis (Batsford, Takamiya & Vogt, 1980).

Twenty percent of the naturally occurring proteins have high isoelectric points. Many of them are present in food (Greenwald, 1976) or are produced by bacteria (Vogt *et al.*, 1983). Once in the circulation these proteins may accumulate in joint structures (Van Lent *et al.*, 1989). Dependent on the degree of accumulation and the (local) immunity against such proteins, this may initiate arthritis or cause a flare of a smouldering arthritis (Lens *et al.*, 1986). Further characterization of cationicity of naturally occurring proteins and their potential to bind to joint structures will provide further insight into which cationic proteins might be potential arthritogens.

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REFERENCES

Apple R.J., Domen P.L. Muckerheide A. & Michael J.G. (1988) Cationization of protein antigens: IV. Increased antigen uptake by antigen-presenting cells. *J. Immunol.* **140,** 3290.

Aston W.P., Ward T.L. & Cooke T.D.V. (1983) The specific *in vitro* antibody mediated retention of bovine serum albumin by porcine hyaline articular cartilage. *Clin. exp. Immunol.* **52,** 280.

Batsford S.R., Takamiya H. & Vogt A. (1980) A model of *in situ* immune complex glomerulonephritis in the rat employing cationized ferritin. *Clin. Nephrol.* **14,** 211.

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 knee joint. *Ann. Rheum. Dis.* **30,** 107.

Cooke T.D., Hurt E.R. & Ziff M. (1972) The pathogenesis of chronic inflammation in experimental antigen-induced arthritis. II Preferential localization of antigen-antibody complexes to collagenous tissues. *J. exp. Med.* **135,** 323.

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.* **22,** 1416.

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

Greenwald R.A. (1976) Connective tissue lysozyme in health and disease. *Sem. Arthr. Rheum.* **6,** 35.

Habeeb A.F.S.A. (1966) Determination of free aminogroups in protein by trinitrobenzene sulfonic acid. *Anal. Biochem.* **14,** 328.

Hollister J.R. & Mannik M. (1974) Antigen retention in joint tissues in antigen-induced synovitis. *Clin. exp. Immunol.* **16,** 615.

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

Jasin H.E. (1975) Mechanisms of trapping of immune complexes in joint collagenous tissues. *Clin. exp. Immunol.* **22,** 473.

Laemli 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. (1984) Quantification of arthritis by ^{99m}Tc-uptake measurements in the mouse knee-joint: correlation with histological joint inflammation scores. *Agents Actions.* **14,** 3.

Lens J.W., Van den Berg W.B., Van de Putte L.B.A. & Zwarts W.A. (1986) Flare of antigen-induced arthritis in mice after intravenous challenge. Kinetics of antigen in the circulation and localization of antigen in the arthritic and noninflamed joint. *Arthr. Rheum.* **29,** 665.

Righetti P.G. & Caravaggio T. (1976) Isoelectric points and molecular weights of proteins. A table. *J. Chromatography,* **127,** 1.

Shen W.C., Yang D. & Ryser H.J.P. (1984) Colorimetric determination of microgram quantities of poly-lysine by trypanblue precipitation. *Anal. Biochem.* **142,** 521.

Teuscher C. & Donaldson D.M. (1979) The deposition and formation of immune complexes in collagenous tissues. *Clin. Immunol. Immunopathol.* **13,** 56.

Van den Berg W.B., Joosten L.A.B., Van de Putte & 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., Kruijsen M.W.M., Van de Putte L.B.A., Van Beusekom H.J., Van der Sluis-van der Pol M. & Zwarts W.A. (1981) Antigen-induced and zymosan-induced arthritis in mice: studies on *in vivo* cartilage proteoglycan synthesis and chondrocyte death. *Brit. J. exp. Pathol.* **62,** 308.

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. **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.

Van den Berg W.B., Van Lent P.L.E.M., Van de Putte L.B.A. & Zwarts W.A. (1986) Electrical charge of hyaline articular cartilage: its role in the retention of anionic and cationic proteins. *Clin. Immunopath.* **39,** 187.

Van Lent P.L.E.M., Van den Berg W.B., Schalkwijk J., Van de Putte L.B.A. & Van den Bersselaar L. (1987) Allergic arthritis induced by cationic antigens: relationship of chronicity with antigen retention and T-cell reactivity. *Immunology,* **62,** 265.

Van Lent P.L.E.M., Van Den Bersselaar, L., Grutters G.J.F. & Van Den Berg W.B. (1989) Fate of antigen after intravenous and intra-articular injection into mice. Role of molecular weight and charge. *J. Rheum.* (in press).

Vogt A., Batsford S., Rodriguez-Iturbe B. & Gracia R. (1983) Cationic antigens in post-streptococcal glomerulonephritis. *Clin. Nephrol.* **20,** 271.

Vogt A., Rohrbach R., Shimizu F., Takamiya H. & Batsford S. (1982) Interaction of cationized antigen with rat glomerular basement membrane: *In situ* immune complex formation. *Kidney int.* **22,** 27.

Wallis W.J., Simkin P.A., Nelp W.B. & Foster D.M. (1985) Intraarticular volume and clearance in human synovial effusions. *Arthr. Rheum.* **28,** 441.