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Experimental Allergic Sialoadenitis

VI. Prevention by Antihistamine and Induction by Intraductal Instillation of Preformed Immune Complexes

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Summary. Experimental allergic sialoadenitis was induced in immunized rats by intraductal challenge with the sensitizing antigen. Intraductal instillation of preformed immune complexes in non-immunized animals caused a similar inflammatory reaction. Treatment of immunized and intraductally challenged rats with antihistamine prevented the development of sialoadenitis. Administration of antiserotonin did not inhibit the inflammatory process. The findings support the contention that deposition of immune complexes in the salivary gland is responsible for the ensuing sialoadenitis.

Instillation of antigen via Stenon's duct into the parotid gland of sensitized rats causes acute sialodenitis (Sela, Ulmansky, Dishon, Rosenmann and Boss, 1972). It has been suggested that the inflammatory process is initiated by antigen-antibody complexes formed within the organ. Further support for the pathogenic role of immune complexes in sialoadenitis has been obtained by intraductal instillation of anti-basement membrane antibodies (Rosenmann, Sela, Ulmansky, Dishon and Boss, 1973) and antiserum to rat plasma (Dishon, Sela, Ulmansky, Rosenmann and Boss, 1973). Immune complexes have been demonstrated immuno-histologically in the parotid glands of sensitized rats challenged with antigen (Ulmansky, Dishon, Rosenmann, Sela and Boss, 1972).

The purpose of the present communication is to further substantiate the part played by immune complexes in the evolution of this model of sialodenitis. Since inflammation follows activation of the complement system (Ward, Cochrane and Müller-Eberhard, 1966) with subsequent release of the mediatory substances (Henson and Cochrane, 1971, Willoughby, Coote and Turk, 1969, Orange and Austen, 1969), treatment with antihistamine and antiserotonin has proved effective in the prevention of the inflammatory process (Cochrane, 1963b, Kniker and Cochrane, 1968). Accordingly, we attempted to impede the development of allergic sialoadenitis by a similar pharmacologic approach. Secondly, sialoadenitis was induced by istillation of preformed soluble immune complexes.

Materials and Methods

Animals. Albino rats of both sexes of the Hebrew University (Sabra) strain, weighing 150–200 gm, and randomly bred local rabbits, weighing about 2,5 kg, were used.

Procedure of Sensitization. Rats were injected subcutaneously on the back with 10 mg of bovine serum albumin[1] (BSA), bovine gammaglobulin[2] (BGG) or ovalbumin[3] (OA) in

1 Bovine albumin, Cohn fraction V, 96–99% albumin, Sigma Chemical Company.

2 Bovine gammaglobulin, Cohn fraction III, approximately 99% gammaglobulin, Sigma Chemical Company.

3 Egg albumin, Nutritional Biochemicals Corporation.

0.5 ml of physiological saline emulsified in 0.5 ml of Freund's complete adjuvant (CFA, Difco). As of the second week, 5 further injections of 20 mg of BSA, BGG or OA in 1 ml of saline were injected intraperitoneally at 3 day intervals. Blood was drawn from the tail vein three days after the last administration. Sera were tested by Ouchterlony's double immunodiffusion technique in agarose gel for precipitating antibodies reacting with the respective antigen.

Preparation of Immune Complexes. Rabbits were given three intramuscular injections, at weekly intervals, of 50 mg of BSA in 2 ml of saline emulsified in 2 ml of CFA. Two further injections of 100 mg of BSA, without adjuvant, were administered during the fifth and sixth week. Blood was drawn by cardiac puncture ten days after the last injection. The serum was inactivated at 56° C for 30 minutes and a quantitative precipitin reaction performed. Immune complexes were obtained at equivalence, centrifuged, washed in buffered saline, and solubilized by the addition of an excess of antigen using an amount equal to 20 times that at equivalence (Cochrane, 1963a).

Instillation of Solutions into the Parotid Gland. A Bardick catheter, 19 gauge, with its stylet in place was introduced into the right parotid duct of anesthesized rats. The stylet was retrieved and 0.5 ml of the appropriate solution were injected without exertion of undue pressure (Ulmansky, Sela, Dishon, Rosenmann and Boss, 1971).

Experimental design: Group 1. One hundred and twenty seven rats, immunized with BSA, BGG or OA, were challenged intraductally with 7.5 mg of the respective antigen in 0.5 ml of saline.

Group 2. Half a milliliter of normal rabbit serum (NRS), BSA, BGG or OA (7.5 mg in 0.5 ml of saline) was introduced into the parotid gland of 56 non-immunized animals.

Group 3. Instillation of 0.5 ml of saline was performed in 53 rats.

Group 4. In twenty-two rats immunized with BSA, an intramuscular injection of chlortrimeton maleate (chlorprophenpyridamine maleate, Schering), 1 mg per 100 gm of body weight, was given 1 hour prior to intraductal challenge with the antigen. The injection of this antihistaminic drug was repeated 2, 6, 10, 14, 18 and 22 hours after the instillation.

Group 5. Six immunized rats were challenged intraductally with BSA. One hour prior to the intraductal instillation of the antigen, the animals were injected intramuscularly with Deseril (1-methyl-lysergic acid butanolamide, Sandoz), 0.05 mg per 100 gm of body weight. The injection of the antiserotonin was repeated 2, 6, 10, 14, 18 and 22 hours after the instillation.

Group 6. Half a milliliter of soluble immune complexes was instilled into the parotid gland of 15 non-sensitized rats.

Histological Examination. The rats were killed 24 hours after the intraductal instillation. The right parotid gland was removed and fixed cold buffered formalin. Paraffin embedded sections were stained with hematoxylin and eosin. The results of the histological examinations were evaluated by the χ^2 test.

Results

Sera of most immunized rats (over 90%) contained precipitating antibodies to the respective antigen (Sela *et al.*, 1972, Ulmansky *et al.*, 1972). The histological alterations of the parotid glands were evaluated semiquantitatively on an arbitrary scale and classified as mild, moderate and severe sialoadenitis, as described previously in detail (Sela *et al*, 1972). Mild sialoadenitis was occasionally observed following instillation of saline. Therefore, in evaluating the results only those cases showing moderate or severe sialoadenitis were considered of significance. Since the histological findings were qualitatively and quantitatively similar in the animals sensitized and challenged with the various antigenic solutions, the results were combined in Table 1. There were no instances of severe sialoadenitis after instillation of saline or proteinaceous solutions in nonimmunized animals (groups 2 and 3). On the other hand, 9 of 56 and 2 of 53 rats presented with moderate sialoadenitis following instillation of proteinaceous solutions and saline, respectively (Tables 1 and 2). Moderate or severe sialoadenitis developed in 69 (54%) of the 127 sensitized rats challenged with the homologous antigen. The difference

Table 1. Incidence of sialoadenitis in experimental and control rats

Groups	1	2	3	4	5	6
Sensitization	BSA, BGG or OA	nil	nil	BSA	BSA	nil
Intraductal instillation of	BSA, BGG or OA	NRS, BSA, BGG or OA	Saline	BSA	BSA	Immune complexes
Treatment	none	none	none	Anti-histamine	Anti-serotonin	none
Number of rats per group	127	56	53	22	6	15
Number of rats with sialoadenitis	69 (54%)	9 (16%)	2 (4%)	0	4 (67%)	11 (73%)

BSA = bovine serum albumin; BGG = bovine gammaglobulin; OA = ovalbumin; NRS = normal rabbit serum.

Table 2. Statistical evaluation of results. Comparison of relevant groups

Groups	2	3	4	5	6
1	$X^2 = 21$ $p < 0.001$		$X^2 = 20.1$ $p < 0.001$	$X^2 = 0.03$ $p > 0.98$	$X^2 = 1.27$ $p > 0.2$
2		$X^2 = 3.3$ $0.1 > p > 0.05$			
3					
4				$X^2 = 12.1$ $p < 0.001$	

in the incidence of adenitis between the latter group (group 1) and that in the non-sensitized rats, the parotid duct of which was instilled with saline or proteinaceous solutions (groups 2 and 3) is statistically significant (Table 2). In addition, it should be stressed that the inflammatory reaction in the sensitized rats was generally more pronounced than that in the non-sensitized animals.

No inflammation was observed in the salivary glands of the 22 sensitized rats challenged with BSA and treated with Chlortrimeton® (Fig. 1). There is a statistically significant difference between this group and that of rats immunized but not treated with antihistamine (Table 2). It is of note that sialoadenitis developed despite treatment with Deseril® in 4 of the 6 rats immunized and challenged with BSA. The difference in the incidence of adenitis between this group and group 1 is statistically not significant (Table 2).

Sialoadenitis developed in 11 of the 15 unimmunized rats, the parotid duct of which was instilled with soluble immune complexes (Fig. 2). The inflammatory

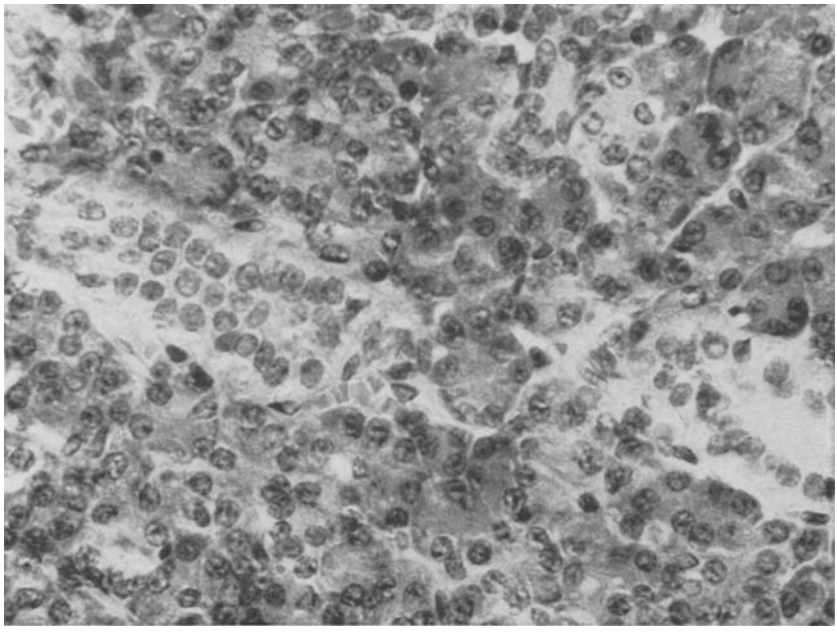

Fig. 1. Parotid gland of sensitized and intraductally challenged rat treated with antihistamine. Note absence of inflammation. Hematoxylin and eosin. × 485

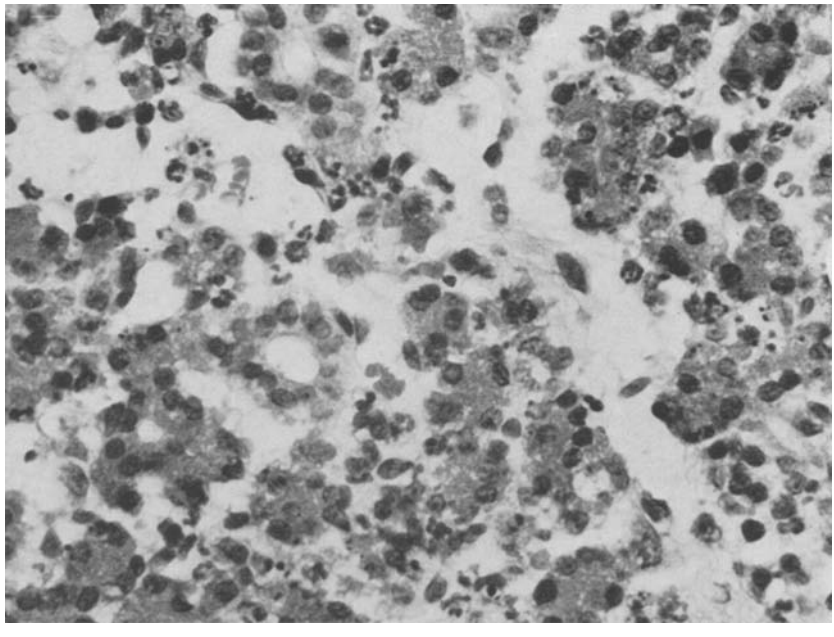

Fig. 2. Parotid gland of non-immunized rat following intraductal instillation of preformed immune complexes. Edema and granulocytic infiltration are conspicuous. Note acinar degeneration and loss and penetration of granulocytes into the acini. Hematoxylin and eosin. × 485

alterations were generally severer than in the other groups and were accompanied by extensive necrosis of acini. There was no statistically significant difference of adenitis between this group and that in immunized rats challenged intraductally with antigen (Table 2).

Discussion

Inflammation develops in the salivary glands of sensitized rats following intraductal challenge with the homologous antigen (Sela *et al.*, 1972, Ulmansky *et al.*, 1972). It has been suggested that the etiopathogenetic mechanism in this model of allergic sialoadenitis is an Arthus-type of reaction, i.e., formation and deposition of antigen-antibody complexes in the gland. This assumption is supported by the immunohistological demonstration of rat gammaglobulin and particularly of the avidity for heterologous complement in the diseased glands, the latter substantiating the presence of immune complexes in a tissue (Burkholder, 1961). In the present study, further evidence for the pathogenic role of such complexes is furnished in as much as an inflammatory reaction follows the intraductal instillation of preformed immune complexes. A similar experimental model was studied by Paronetto *et al.* (1962), who produced acute hepatitis by introducing immune complexes into the bile duct of the rat. Subsequent to complement activation by immune complexes, the mediatory substances of inflammation, primarily histamine, are released (Cochrane 1963b, Willoughby *et al.*, 1969, Orange and Austen, 1969). Henson and Cochrane (1971) have shown that histamine is also liberated as a result of the leukocyte dependent reaction in the presence of antigen and antibody. Accordingly, in our experiments, antihistamine medication inhibited the inflammation, which otherwise followed the intraductal challenge of the parotid gland in sensitized rats. Administration of antiserotonin, on the other hand, did not prevent the development of sialoadenitis. Our results, therefore, indicate that histamine release is the more important factor in triggering the inflammatory reaction in the salivary glands of the rat, supporting the notion that the amount of the released serotonin contributes but little to the initiation of inflammation (Humphrey and White, 1970). It is interesting that Kniker and Cochrane (1968) had to use the combination of antihistamine and antiserotonin to completely inhibit immune complex glomerulonephritis in the rabbit. The discrepancy between their and our findings in this respect might be best explained by the different species used and the different target organ studied.

The literature pertaining to immune sialoadenitis has been previously reviewed (Sela, *et al.*, 1972, Ulmansky *et al.*, 1972). The salivary glands, just as other organs, are also prone to injury by either autoimmune processes (Haferkamp, 1962) or the deposition of immune complexes in the glandular parenchyma.

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