Effect of Injury on Antigenic Composition of Rabbit Skin (34888)

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Previous reports from this laboratory have described a chronic, remittent, nodular lesion in the dermal connective tissue of rabbits produced by a single intracutaneous injection of group A streptococcal cell wall fragments (1). The cell wall material induces characteristic histological alterations including disruption of collagen (2). The present investigation was undertaken to determine whether this chronic injury is reflected in changes in the antigenic composition of the tissue. Guinea pigs were immunized with several fractions of extracts of normal rabbit skin, or of skin injured with streptococcal cell wall fragments, and the antisera were used to characterize the antigenic composition of such extracts. A distinctive antigenic component characteristic of injured connective tissue is shown, but it is not unique to streptococcal lesions.

Materials and Methods. Cell wall fragments were obtained from group A streptococci (3). Lesions were induced in New Zealand white rabbits by intradermal injection of 0.5 mg of cell walls suspended in 0.2 ml of physiological saline. Cell walls were resonicated for 10 min just before use. For extractions, full-thickness skin lesions were collected and trimmed free of normal skin. Cell wall-induced lesions were collected 4 to 12 days following injection at the time of apparent maximum tissue involvement. The animals were closely shaven and rinsed with alcohol before collection of samples.

Lesions were induced with other agents including turpentine (Terp), 0.1 ml/site; Freund's complete adjuvant (FA) 0.1 ml/site; lipopolysaccharide endotoxin (Tox), 0.1 mg/site (Difco Laboratories); and guinea pig antirabbit skin serum (ABS-Les), 0.2 ml/site. All injections were made intradermally and involved tissue was collected 4 to 6 days following injection.

Skin fractions were prepared by modifications of a method previously described (4). Skin samples were snap frozen by dipping in liquid nitrogen, and pulverized with a stainless steel piston driven with a small sledge hammer. The tissue powder was extracted sequentially with three buffers in the following order: (a) phosphate buffered saline (PBS), ph 7.0, 0.1 ionic strength, plus 0.05 *M* NaCl; (b) phosphate buffer, pH 7.7, plus 0.35 *M* NaCl to give 0.45 ionic strength (BS fraction used in most of these studies); (c) 0.5 *M* citrate buffer, pH 3.6. Extractions were made overnight at 4° with continuous agitation. Following each extraction, the solutions were centrifuged at 37,000g for 60 min at 4° and the residue was used for the subsequent extraction. The insoluble residue following all of the extractions was also collected. Each fraction was dialyzed against distilled water at 4°. The precipitates which formed were collected by centrifugation. Following dialysis all fractions were lyophilized. Prefixes serve to designate normal (N) or lesion (L) origin of an extract. Each fraction was analyzed for nitrogen, tyrosine, hydroxyproline, uronic acid and amino sugar. The chemical analysis and yields of the fractions were similar for normal and lesion samples and were comparable to previous results (4).

Guinea pigs were immunized with the skin fractions by injection of 1-mg samples given subcutaneously and intraperitoneally. Four weekly injections were given, followed by a rest of 4 weeks and a second series of immunizations. The first two injections were given with Freund's complete adjuvant. The animals were bled 1 week after the last injection.

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carried out in 0.75% Ionagar in borate buffer pH 8.6, 0.05 ionic strength. Immunoelectrophoresis was done with 1% Ionagar in barbital buffer, pH 8.6, 0.1 ionic strength. Antigens were lyophilized samples suspended in physiological saline. Skin fractions and leukocytes were tested at a concentration of 10 mg/ml. Serum was tested whole and diluted. A commercial rabbit gamma G globulin (R7S) was used (Immunology, Inc.).

Serum absorptions were performed by suspending lyophilized antigen in the serum, incubating at 37° for 1 hr and at 4° overnight. The serum was centrifuged at 10,000*g* for 30 min at 4°. Pooled antigen preparations and preparations from individual donors were used independently in absorption studies.

Passive hemagglutination of sheep erythrocytes was performed by the method of Kabat and Mayer (5) using microtiter equipment. All antisera were heat inactivated and absorbed with half volumes of packed sheep erythrocytes before use. The antigen concentration for sensitization of tanned red cells was 1 mg/ml. Controls included preimmune guinea pig serum.

Rabbit PMN lysosome fractions, hyperimmune guinea pig antiserum to rabbit polymorphonuclear leukocytes (APMN) and antiserum to rabbit alveolar macrophages (AMac) were donated by Dr. John Spitznagel and Dr. Marlene Absher. Specific and cross-absorbed goat antisera to rabbit IgG, IgA, and IgM were kindly donated by Dr. Michael Potter.

Results. Numerous preliminary studies by immunodiffusion and immunoelectrophoresis with antisera to the 7 fractions obtained from normal rabbit skin revealed that these extracts collectively contain at least 19 distinct antigens, at least 7 of which are also present in rabbit serum. When the reactions of these antisera with normal and lesion extracts were compared, the antisera to the BS fractions (basic high salt pH 7.7, ionic strength 0.45) were the only sera capable of detecting differences between the normal and lesion extracts. For this reason, the BS fraction was chosen for further characterization. A second reason for interest in this material was a greater toxicity of antisera to this fraction when introduced into the skin of rabbits (Jones and Schwab, unpublished observations).

TABLE I. Antigens of Rabbit Skin BS Fraction.

Antigen[a]	Occurrence[b]
A	Connective tissue
B	Connective tissue lesions
C, C′	PMN, skin lesions
D	PMN, skin lesions
E	PMN, skin lesions
F	Serum (IgG), skin
G	Serum (Prec),[c] skin
H	Serum (Sup),[c] skin
I	Serum (Prec), skin
J	Macrophages, skin

[a] Precipitin are showing nonidentity with others listed.

[b] Detected by direct or indirect means.

[c] Fraction of 50% $(NH_4)_2SO_4$ saturation.

Ten antigens were defined in the BS fraction of group A cell wall lesion extracts by immunodiffusion or immunoelectrophoresis. The identification of these is summarized in Table I and Fig. 1. Pooled antiserum against the BS fraction of cell wall lesion (PAPL) developed 4 lines with pooled lesion BS fraction (PLBS) and one with pooled normal BS fraction (PNBS). This latter precipitin line showed identity with a line developed with pooled antibody against normal BS fraction (PAPN).

The reaction of antibody against normal BS fraction with normal or lesion antigen could be abolished by treating the fraction with 0.25% trypsin at pH 7.3 or 7.7 for 30 min at 37°. The reaction was not affected by treatment with 0.2% collagenase at pH 7.3 or 0.03% hyaluronidase at pH 7.7 for 60 min.

Three of the lesion antigens originated from polymorphonuclear leukocytes (PMN). Antibody against lesion BS fraction revealed 2 lines with PMN. One gave a line of identity with anti-PMN, and the other showed a spur (Fig. 2). Anti-PMN showed 3 lines with lesion BS fraction (PLBS) but the middle line was faint. Two of these lines showed identity with PMN antigens.

Antilesion BS fraction developed one faint line with alveolar macrophages which showed

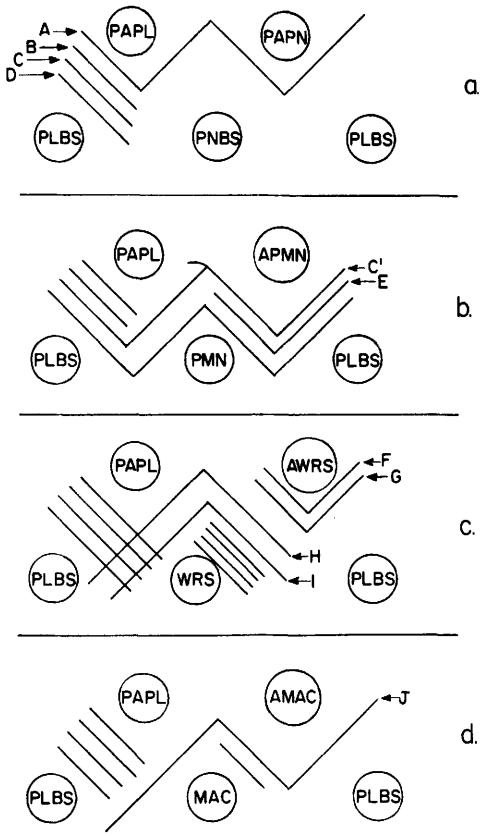

FIG. 1. Summary of the precipitating antigens of rabbit skin BS fraction showing their position in double immunodiffusion. These antigens are listed in Table I. PAPN = pooled anti-pooled normal skin BS fraction, PAPL = pooled anti-pooled lesion BS fraction, APMN = antirabbit polymorphonuclear leukocyte, AWRS = antiwhole-rabbit serum, AMAC = antirabbit macrophage, PNBS = pooled normal BS fraction, PLBS = pooled lesion BS fraction.

identity with a line developed with goat anti-macrophage serum. Antilesion BS fraction did not reveal this antigen in the homologous extract, which was probably due to the small amount in the preparations. Antimacrophage serum also exhibited a weak reaction with normal skin extract.

Four of the 10 antigenic components of the BS lesion fraction were also identified in serum. Several serum components were detected in the BS extracts by antirabbit serum (AWRS) and antisera to skin extracts reacted with at least two serum components (lines H, I). Both of these components exhibited identity with serum components revealed with AWRS. Antisera to skin fractions did not react with rabbit IgG but this antigen was revealed in extracts of normal skin and lesions with goat anti-IgG (line F). Rabbit IgA or IgM was not detected in the BS preparations. Since the BS fractions were made secondarily to an overnight extraction with an excess of neutral saline, the IgG detected may represent immunoglobulin more tightly bound to the connective tissue. The significance of this property is not yet clear. Attempts to absorb the IgG onto the insoluble residue were unsuccessful.

Figure 3 shows that antinormal BS fraction revealed the same common line in extracts of all the lesion types tested. Antilesion BS fraction revealed 4 lines of identity between BS fractions of cell wall lesions, turpentine lesions (Terp) and endotoxin lesions (Tox). Reactions with extracts of Freund's adjuvant lesions (FA) were similar but less distinct. Antilesion BS fraction revealed only a single line with extracts of normal skin or lesions induced by injections of antibody to normal BS fraction (ABS-Les).

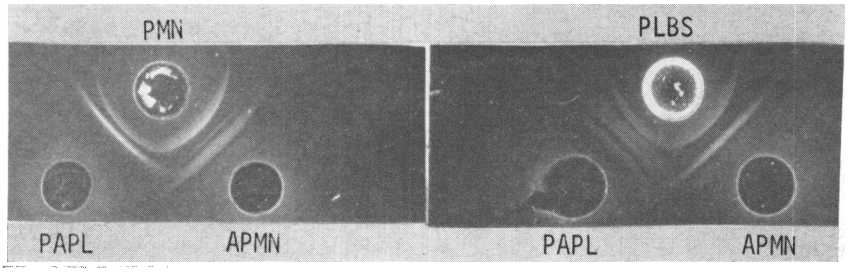

FIG. 2. Immunodiffusion patterns showing relationship of antigens from polymorphonuclear leukocyte (PMN) and BS extracts of cell wall lesions (PLBS). The pattern on the left exhibits a spur which developed when PAPL and APMN were tested simultaneously with PMN extracts.

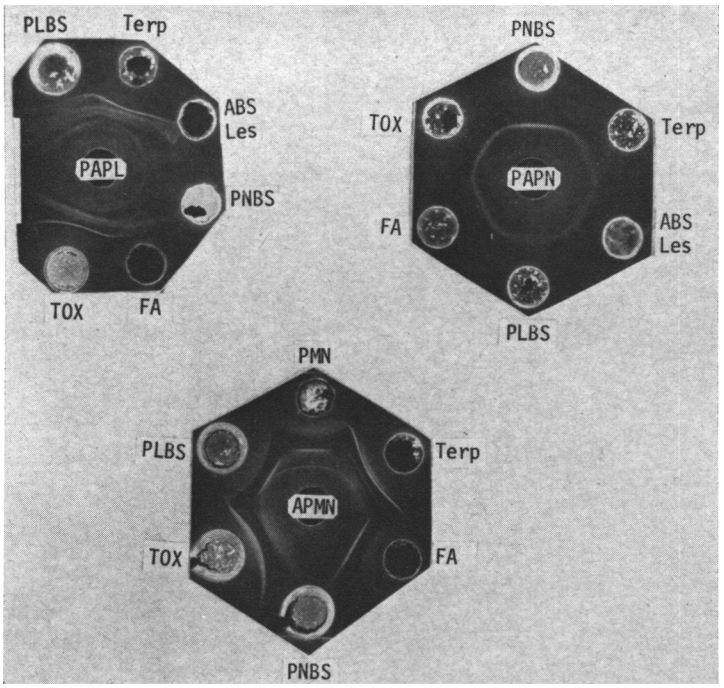

FIG. 3. Immunodiffusion patterns of PAPL, PAPN and antirabbit polymorphonuclear leukocyte serum with BS extracts of several types of lesions: Terp = turpentine; ABS-Les = passive antibody; FA = Freund's complete adjuvant; Tox = endotoxin.

When tested with antirabbit polymorphonuclear leukocyte serum (APMN), PMN antigens were detected in each of the extracts except those of normal skin. As anticipated, the greatest reactivity was observed with the turpentine lesion extracts.

Guinea pig antibodies to pooled BS fractions of normal skin or skin lesions, or to extracts of skin samples from individual animals, were measured also by passive hemagglutination. Individual antisera to individual extracts were similar to those of pooled antisera to pooled extracts. Antisera to lesion material were highly selective for lesion extracts and had little or no tendency to react with extracts of normal skin. Antisera to normal skin extracts were less discriminating and frequently of lower titer. The antibody responsible for distinguishing lesion extracts was against polymorphonuclear leukocytes since lyophilized or whole viable PMN completely absorbed the activity of antilesion BS for lesion BS fraction. The lysosome fraction of PMN was less effective. Polymorphonuclear leukocytes did not absorb the activity of antinormal BS fraction for the homologous antigen.

Low titers of antilesion BS fraction with cell walls or pooled antistreptococcal vaccine with lesion BS fraction were observed, but this was also true of extracts of normal skin. Absorption of 1 ml of antilesion BS with 20 mg of lesion BS fraction removed all activity, while absorption with five times this concentration of insoluble cell wall fragments of group A, A variant, group A mucopeptide, or cell walls in combination with normal BS material failed to reduce the hemagglutinating titer.

Discussion and Summary. In discussion of autoimmunity there is frequently a suggestion that appropriate injury of tissue can result in the appearance of new antigenic structures recognized as foreign by the host (6, 7). It has been reported also that tissue structures can be rendered immunogenic when complexed with bacterial components (8). The group A streptococcus has been especially impli-

cated. The present studies were designed to detect an antigenic component in rabbit dermal connective tissue, injured with group A streptococcal cell wall fragments, which reflects either a modified tissue structure or a complex of tissue and bacterial antigen.

The results demonstrate that a "new" antigenic structure (line B) is formed in injured connective tissue; however, this component is not unique to group A streptococcal cell wall lesions since it is also found in lesions induced with endotoxin, Freund's adjuvant, or turpentine. This antigen is not detected in serum, PMN, PMN lysosomes, alveolar macrophages, spleen cells, or extracts of normal skin. The further characterization, distribution, and possible function of this component has not been done. We have been unable to detect an autoimmune response against this or any other component of rabbit skin (Jones and Schwab, submitted for publication). The spur line (C′ to C) observed with antilesion BS fraction and anti-PMN when tested with PMN may also represent a modification (loss) of a PMN antigenic structure in connective tissue lesions.

The experiments described here were made with pooled antisera and pooled antigens, but serum from individual guinea pigs immunized with extracts of individual rabbit tissue gave similar results. The possible confusion of isoantigenic differences between rabbits was controlled by comparing normal and lesion skin tissue taken from opposite flanks of the same animal. Lesion antiserum was selective for lesion extracts in passive hemagglutination tests. The antigens involved were derived from PMN since absorption with PMN abolished the reaction. However, in immunodiffusion tests such absorbed serum still reacted with BS fractions of lesion and normal skin.

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