STUDIES ON A TOXIC CELLULAR COMPONENT OF GROUP A STREPTOCOCCI

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The hemolytic properties of extracts of sonic disrupted group A streptococcal cells have been described previously by Schwab (1956a, 1956b). After a single intradermal injection into rabbits, similar cellular extracts have been observed to induce chronic remittent nodular skin lesions associated with marked changes in the dermal connective tissue. It is the purpose of this communication to describe these skin changes along with observations concerning certain properties of the active material in these extracts.

MATERIALS AND METHODS

Extraction of cells. The cultivation, harvesting, and extraction of the organisms has been described previously (Schwab, 1956a). In the experiments reported here the washed cells from 1 L of an 18 hr broth culture were suspended in 20 ml of a pH 7.0, 1/2 0.15 phosphate buffer and subjected to sonic vibration in a Raytheon 9 Kc sonic oscillator for periods of 1/2 to 3 hr. The material was then centrifuged in a Spinco preparative centrifuge at 36,000 × G for 30 min, the supernatant fluid filtered through a Selas 02 filter and frozen at −20 C in sealed ampules. The sterility of each preparation was confirmed by inoculating thioglycolate broth and blood agar plates with 0.5 ml aliquots. The initial bacterial suspensions, prior to sonic disruption, contained from 5.0 to 6.0 mg cell N per ml (this corresponds to about 1 × 10^1^ coci per ml, as determined in the Petroff-Hausser bacterial counting chamber). Following 2 hr of sonic vibration, the centrifuged, filtered extract contained 3.6 mg N per ml, 10 per cent of which was not precipitable with trichloracetic acid. Thus, on the basis of nitrogen determinations the extracts contained about 2 per cent protein. Nitrogen values were determined by the Kjeldahl technique.

Organisms. The D-58 strain of a type 3 group A streptococcus (Streptococcus pyogenes) was the organism used except when otherwise indicated. Other group A streptococci employed were type 3, strains C203U and C203S obtained from Dr. Hutton Slade; a type 11 Blackmore strain obtained from Dr. Allan Bernheimer; and type 12 and type 4 strains from the authors' collection. Other streptococci used include group B, Streptococcus mastitidis; group B, Streptococcus agalactiae, ATCC strain 7077; group C, Streptococcus equi, ATCC strain 9527; group D, Streptococcus durans, ATCC strain 9810; and Streptococcus mitis, ATCC strain 9811.

Animals. New Zealand white rabbits each weighing 2000 to 2500 g were used throughout. Sections for histological study were fixed in alcohol and formalin. Paraffin sections were stained with hematoxylin and eosin.

RESULTS

Effects of a single intradermal injection of an extract of group A, type 3, D-58 strain of streptococcus. Thirty-eight rabbits were injected intradermally at a single site in the flank, each with 0.2 ml of the extract of strain D-58. Thirty-four animals were observed for 10 days; 16 animals for 21 days; and 12 animals for 30 days. Selected rabbits were killed for histological study at various intervals. During the first 48 hr after injection a slight thickening and erythema of the skin was noted at the site of injection in 20 animals. These lesions varied in size from 20 by 30 to 40 by 80 mm and were elevated about 1 mm above the skin surface. In a majority of the rabbits this slight erythema and edema subsided completely in 12 to 24 hr.

What were considered to be the characteristic and unique macroscopic features of the reaction to the extract developed later, after the slight

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edema and erythema at the site of injection had subsided. Multiple nodules of the skin developed in all animals at various intervals after injection; in 2 animals during the second day, in 25 animals during the third or fourth day, in 10 animals during the fifth or sixth day, and in a single animal during the thirteenth day. The size of the total skin area involved varied from 50 by 30 to 120 by 80 mm. The major area of involvement was anterior, posterior, and ventral to the site of injection; however, a number of animals exhibited nodules dorsal to the site of injection. The individual nodules varied markedly in size and shape and distribution. Figure 1 illustrates the most frequent type of initial nodular lesion. The most common nodules were rounded, projected from 2 to 15 mm above the skin surface and measured 8 by 8 to 30 by 40 mm. The number of distinctly separate nodules noted in a single rabbit varied from 2 to 46. In addition to rounded nodules, elongated ridgelike elevations that measured about 15 mm in width, 30 to 50 mm in length and projected about 15 mm above the skin surface, developed in some animals. Figure 2 illustrates a lesion of this type. Occasionally these elongated ridgelike elevations formed circular lesions. Frequently the isolated nodules increased in size, coalesced, and formed elevated nodular plaques that measured up to 60 by 80 mm and projected 10 to 15 mm above the skin surface. Nodules that are beginning to coalesce are illustrated in figure 3. The individual nodules reached a maximum size 48 to 72 hr after appearance. At this time they were moderately soft in consistency with marked erythema of the overlying skin. Spontaneous hemorrhage into the lesions was not a feature of the reaction. However, slight trauma would produce hemorrhage into the lesions. Necrosis of the skin was not noted.

After the initial nodular lesion reached a maximum size, the subsequent course of the reaction in 12 animals that were observed for 30 days was quite variable. In 2 animals the lesions appeared to completely subside in 10 to 12 days and no subsequent lesions developed. In 3 animals the acute edematous reaction subsided in 4 or 5 days leaving firm, nodular, slightly elevated, plaquelike lesions which persisted until the experiment was terminated. In 7 animals definite remissions and exacerbations of the skin lesions were observed. In 4 of these animals the initial nodular process appeared to completely subside after 6 to 10 days. In 3 of the 7 animals the initial lesions subsided after 6 or 7 days, leaving indurated, pale, plaquelike areas that measured from 30 by 40 to 60 by 80 mm and which were elevated 2 to 4 mm above the skin surface. In all 7 animals new lesions similar to the initial nodular reaction appeared in the same skin area in which the initial reaction had either completely or markedly subsided. These exacerbations were noted between 4 and 21 days after abatement of the initial lesions. Figure 4 shows a lesion which developed in the previously involved area after the initial nodular reaction had subsided. In general the lesions associated with the exacerbations were somewhat smaller and there were fewer separate nodules. Otherwise they were quite comparable to the initial nodular reactions described above and they subsided in a similar manner.

Microscopic studies revealed a wide variety of alterations. A complete description and interpretations of the histological changes, along with histochemical studies, will be reported elsewhere; however, the general character of these reactions may be described as follows: Alterations were noted in all layers of the dermis, although they were more marked in the deep layers. The initial reaction noted 24 hr after the appearance of lesions, consisted of separation of the collagen bundles and infiltration by an acute inflammatory exudate. Changes noted between the fourth and
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Figures 1-4
seventh day after injection are shown in figures 5 to 7. At this stage the most extensive change consisted of areas in which the connective tissue and the leukocytes that had infiltrated the area appeared necrotic (figures 5 and 6). In other areas the individual collagen bundles appear swollen (figure 7). In scattered areas there were dense accumulations of necrotic appearing mononuclear and polymorphonuclear cells. Scattered throughout these focal accumulations of cells were masses of homogenous staining material that appeared to be remnants of markedly altered collagenous tissue. The reaction noted 30 days after injection and 27 days after the appearance of the lesion is illustrated in figure 8. In general the histological changes seen 21 to 30 days after injection were characterized by focal accumulations of mononuclear and giant cells in some areas and rather diffuse infiltrations of similar cells in other areas. In some sections foci of scarring were seen, along with evidence of active fibroplasia. In the same sections that showed evidence of a chronic reaction there were small areas that appeared to be foci of acute necrosis. In the early lesions there was involvement of scattered blood vessels by an acute inflammatory process; however, this change did not appear to be the primary alteration with the other changes being secondary to vascular occlusion. The toxic material appeared to have a direct effect on connective tissue of the dermis.

Extracts of other streptococci. Extracts of group A organisms belonging to types 12, 11 and 4; 2 strains of type 3, as well as streptococci of groups C and D; 2 strains of group B; and a viridans organism—S. mitis, S. equi, and the group B strains. The typical multinodular lesion developing 2 or more days after injection, as described above, was observed with all extracts of group A organisms. This was observed with the D-58 strain in 12 of 12 animals; with type 4 in 6 of 6; with C203S strain in 5 of 6; the Blackmore and C203U strains in 4 of 6. With the type 12 extract only 2 of 6 injection sites displayed typical nodular lesions, and these developed after 13 days. In 3 of the 12 areas injected with a group B extract questionable nodular lesions were observed. These injections were on the same flank and posterior to the control injection of a group A strain. To determine if this reaction at the group B injection site was confused with, or influenced by, the proximity of a group A extract, 7 rabbits were injected at a single site with only extract of group B S. agalactiae. One of these animals developed a nodular lesion.

Thus, it may be concluded that many strains of group A streptococci contain a toxic cellular component that will induce a nodular lesion in the skin of rabbits and, with the possible exception of group B, it is not a component of the other streptococci tested.

Effect of heat. An extract of strain D-58 was held at 56°C for 30 min and 0.2 ml injected in the flank of each of 6 rabbits. The opposite flank was injected with 0.2 ml of unheated control material. All rabbits developed typical nodular lesions on both flanks within 3 days.

This experiment was extended, holding the sonic extract, in sealed ampules, in a boiling water bath for periods of 5 and 30 min. The heavy

Figures 5-8. Photomicrographs of skin lesions in rabbits following a single injection of a toxic cellular component of group A streptococci.

Figure 5. Lesion 7 days after injection and 4 days after appearance of the lesion. Area of necrosis of connective tissue of the dermis and diffuse infiltration by an acute inflammatory exudate. × 100.

Figure 6. Lesion 7 days after injection and 4 days after appearance of the lesion. Area of necrosis of connective tissue. × 430.

Figure 7. Lesion 4 days after injection and 48 hr after appearance of the lesion. Swelling of bundles of collagen fibers of the dermis and infiltration by leukocytes. × 430.

Figure 8. Lesion 30 days after injection and 27 days after appearance of the lesion. Focal accumulation of mononuclear and giant cells. × 430.
precipitate which formed was removed by centrifugation at 1100 × G in the refrigerated centrifuge. Each supernatant, as well as an unheated control, was injected into 3 sites on 6 rabbits. All animals were observed for 3 weeks. In 5 animals a typical reaction occurred at and below the injection site of the control material. None of the sites receiving extract heated at 100 C developed typical nodular lesions. However, in 2 of the rabbits localized lesions about 25 by 25 mm developed at injection sites of the heated material. Thus, temperature of 100 C markedly reduces activity of the material responsible for the nodular lesion.

Titration of extracts of cells subjected to 30 min and 3 hr periods of sonic vibration. An attempt was made to determine the relative amount of the active material in an extract, and to establish whether the amount of active material released from the cells is dependent on the period of sonic vibration, as has been demonstrated for the homolysin released from the cells (Schwab, 1956c). A suspension of cells was prepared as usual and after 30 min of exposure to sonic vibration, 4.0 ml was removed and the remainder further treated for a total of 3 hr. Dilutions of 1/1, 1/10, 1/100 and 1/500, were made in pH 7 phosphate buffer. Each dilution was injected into 2 rabbits in a volume of 0.2 ml. The extracts of 1/2 and 3 hr treatments were introduced in opposite flanks. Rabbits were observed for 3 weeks. Typical multinodular lesions developed in 3 days on both flanks in all rabbits given undiluted or 1/10 extracts. Lesions of this type were not observed in the other animals. A single 15 by 15 mm nodule developed between 9 and 15 days at the site of injection of the extract prepared with 3 hr sonic vibration in 1 animal receiving 1/100 and 1 receiving 1/500 dilutions. It may be concluded that a 30 min period of sonic oscillation releases a significant amount of the active material from cells. The crude method of titration does not permit precise comparison of amount of active material released from cells subjected to various periods of sonic treatment.

Effect of rabbit antiserum. Antiserum against the crude extract was prepared by immunizing rabbits with 5 subcutaneous injections of 1.0 ml each of the type 3, D-58 extract plus alum adjuvant, at weekly intervals. Following a 4-week rest, 11 additional injections of 1.0 ml each of the extract without adjuvant were given at 3-day intervals. The animals were bled 10 days after the last injection.

Four ml of serum was mixed with 0.2 ml of homologous extract and held at 30 C for 30 min and then kept at 3 C for 24 hr. The resulting precipitate was removed by centrifugation at 1100 × G for 30 min at 3 C. Six rabbits were injected with 0.4 ml of the supernatant, which was equivalent to 0.2 ml of a 1/10 dilution of the sonic extract. These rabbits were injected at 2 other sites with comparable amounts of extract plus normal rabbit serum and extract plus pH 7 phosphate buffer. A fourth site received the antigen-antibody precipitate resuspended in 4.0 ml buffer.

All animals developed typical nodular lesions within 3 to 23 days at control sites injected with extract plus normal serum and extract plus buffer. The resuspended precipitate induced a persistent nodule about 15 by 15 mm at the injection site. The area receiving extract plus antiserum remained negative in 3 animals. The other 3 rabbits developed nodules at this site 13 to 32 days after injection. From this preliminary experiment it appears that antiserum modifies the active factor in the extract. Further interpretation must await purification of the active component.

DISCUSSION

A large number of cellular components and extracellular products of group A streptococci have been described. McCarty (1952) has reviewed the biological properties of these substances and their possible role in streptococcal infections. None of these previously described products has been shown to produce reactions similar to those reported here. Erythrogenic toxin will produce a lesion in the skin of rabbits (Fraser and Plummer, 1930). However the lesion is distinctly different from the multinodular remittant lesion described above. Stetson (1956) has recently described the properties of lysates of group A streptococci. A single injection of these lysates produced a reaction in the skin of rabbits of maximum size and intensity at about 24 hr after injection and subsiding over 2 or 3 days; again a reaction quite different from that described above.

It should be emphasized that the reactions described in this report were produced in normal rabbits after a single injection of a sterile extract.
of group A streptococci. This is in contrast to the many previous studies of Swift and his associates which have been reviewed (Swift, 1949). These workers utilized killed or living whole cells of a variety of streptococci to induce reactions in the skin of rabbits. Both the experimental method used and the lesions produced were definitely different from those described here. The very moderate secondary reaction that followed injection of "non-hemolytic" or "viridans" streptococci was not noted after injection of "typical hemolytic streptococci" which were probably, group A organisms.

A striking feature of the reaction under consideration was the development of widely separated nodules following injection of the sterile extract at a single site. The distribution of the separate lesions did not suggest that the spread of the toxic material was along lymphatic channels or that the spread was the result of the effect of gravity alone. A number of separate nodules developed dorsal to the injection site. The mechanism of spread has not been defined.

Another remarkable aspect of the reaction was the remissions and exacerbations which occurred over a period of at least 30 days following the single injection of a sterile extract of group A streptococci. The nodules associated with the exacerbations appeared in the previously involved skin areas. In some animals the process was remittent while in others it appeared intermittent, as determined by macroscopic observation. Until additional data are available it would seem of little value to speculate on the mechanism responsible for this reaction.

It is believed that further characterization of this toxic cellular product of group A streptococci may permit the development of experimental models useful for the study of disorders associated with these organisms.

SUMMARY

Remittent multinodular lesions of dermal connective tissue developed in rabbits following a single intradermal injection of a soluble extract of group A streptococcal cells subjected to sonic vibration. Extracts of streptococci other than group A, prepared in a similar manner, failed to induce this remittent multinodular lesion. The activity of the toxic material was markedly reduced by a temperature of 100 C for 5 min and appeared to be modified by combination with antisera. It seems likely that further characterization of this toxic material may be a useful approach to the study of the mechanism of tissue damage in group A streptococcal infections.

REFERENCES


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