Electrophoretic and Immunoelectrophoretic Studies of Sera from Normal, Tuberculous, and Noninfected Tuberculin-Sensitive Guinea Pigs

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ABSTRACT

DARDAS, T. J. (Michigan State University, East Lansing), and V. H. MALLMANN. Electrophoretic and immunoelectrophoretic studies of sera from normal, tuberculous, and noninfected tuberculin-sensitive guinea pigs. J. Bacteriol. 92:76–81. 1966.—Normal guinea pig serum was separated into seven fractions by electrophoresis on cellulose acetate membranes. Thirty antigens were found by immunoelectrophoresis: albumin, 6 α1 globulins, 11 α2 globulins, 5 β1 globulins, and γ globulin. Hyper-α2-globulinemia was detected in sera from guinea pigs 14 days after inoculation with viable virulent Mycobacterium bovis. An additional α2 globulin, not demonstrable prior to infection, was detected concomitantly with the hyper-α2-globulinemia by immunoelectrophoresis. The additional α2 globulin was tentatively named α2-T. It persisted until the death of the guinea pigs. Neither hyper-α1-globulinemia nor the α2-T antigen was detected by cellulose acetate electrophoresis and immunoelectrophoresis of sera from guinea pigs sensitized with heat-killed M. bovis. Both changes were due to the disease, not to delayed sensitivity alone.

A common aim of many studies of disease in experimental animals is to disclose features of pathogenesis common to the natural disease in man and animals; similarities can then be studied in greater detail to understand better the disease process. Many attempts have been made to detect specific antibodies, antigens, or other substances in the sera of infected individuals of pathognomonic significance for tuberculosis, but the results have been disappointing. Variations in serum proteins during the disease have been examined by electrophoresis (6); however, the changes are nonspecific and resemble quite closely those that occur in a variety of other bacterial and viral diseases (2).

Hypoalbuminemia and hyperglobulinemia have been detected in tuberculous guinea pigs by zone electrophoresis, (5, 9, 10, 11). Because of the greater resolution of serum proteins by immunoelectrophoresis, it was felt that additional information could be obtained regarding these changes by use of this procedure.

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MATERIALS AND METHODS

Infection and sensitization of guinea pigs. Fifty-two male guinea pigs, approximately 6 months old and weighing approximately 300 g each, were divided into 13 groups of four per group. Serum was obtained from blood collected aseptically from all of the guinea pigs prior to infection or sensitization. The strain of Mycobacterium bovis was isolated in 1960 and identified by growth and morphological characteristics, cytochemical tests, virulence for laboratory animals, and allergenicity for guinea pigs (7): 1 mg injected intradermally into one calf and three swine caused disseminated gross and microscopic lesions; 0.01 mg (wet weight) injected intraperitoneally into guinea pigs consistently caused disseminated gross and microscopic lesions, and death in approximately 45 days.

Each guinea pig in groups one through nine was inoculated intraperitoneally with 0.01 mg (wet weight) of M. bovis. The four guinea pigs in different groups were bled from the heart at 7, 14, 21, 28, 33, and 41 days after inoculation.

Fourteen guinea pigs were inoculated intraperitoneally three times with 1.0 mg (wet weight) per inoculation of heat-killed M. bovis (100 C, 30 min) at 3-day intervals. Fourteen days after the first inoculation, two guinea pigs were inoculated intradermally with 0.1 ml of tuberculin (PPD-S, 1 strength, Parke, Davis & Co., Detroit, Mich.), and observed at 24 and
48 hr; the 10-mm diameter of induration at 48 hr was recorded. Four guinea pigs in groups 10 through 12 were bled at 15, 22, and 29 days, respectively, after inoculation with heat-killed M. bovis.

*Anti-guinea pig serum.* Portions of the preinoculation guinea pig sera were pooled and precipitated with alum (4). Five adult Dutch Belted rabbits were inoculated intramuscularly with the alum-precipitated antigen and were bled 5 days after a single intraperitoneal injection of untreated guinea pig serum. The antisera were tested individually and pooled.

*Cellulose acetate membrane electrophoresis.* Samples of 2.5 μl of fresh serum were subjected to electrophoresis for 2 hr, at 4°C, with a current of 1 mA per strip. A barbital-acetate buffer at pH 8.6 was used (8). After electrophoresis, the proteins were stained with Ponceau S and examined in a densitometer.

*Immunoelectrophoresis.* Immunoelectrophoresis was performed (4), and the immunoprecipitates were stained with protein-, lipid-, and carbohydrate-specific stains (3).

**RESULTS**

*Analyses of normal guinea pig sera.* Normal sera were separated into seven components by electrophoresis on cellulose acetate (Fig. 1A). These included albumin, one α1 globulin, one α2 globulin, one β1 globulin, two β2 globulins, and γ globulin. A prealbumin fraction was resolved with a tris(hydroxymethyl)aminomethane-ethylendiaminetetraacetic acid-boric acid buffer (1). Thirty antigens were detected in normal serum examined by immunoelectrophoresis: albumin, 6 α1 globulins, 11 α2 globulins, 6 β1 globulins, 5 β2 globulins, and γ globulin. A representative immunoelectrophoretogram is shown in Fig. 2. The immunoprecipitate pattern varied slightly

![Fig. 1. Typical densitometric recordings of cellulose acetate membranes after electrophoresis of sera from normal (A) and tuberculous guinea pigs at 7 (B), 14 (C), 21 (D), 28 (E), and 33 (F) days postinoculation with Mycobacterium bovis.](http://jb.asm.org/Downloaded from http://jb.asm.org/)
from serum to serum, and most of the precipitates exhibited some variation in displacement and clarity. The lines formed by albumin, $\alpha_1-4$, $\alpha_1-5$, $\alpha_2-2$, $\alpha_2-6$, $\beta_1-1$, and $\gamma$ globulin were generally found in the same relative position.

Albumin formed the largest and most anodic precipitate in the immunoelectrophoretogram. At least six $\alpha_1$ globulins were found beneath the curvature of the albumin precipitate. Spatial variations sometimes occurred, and all of the lines were not always present simultaneously. The $\alpha_1-4$ and $\alpha_1-5$ globulins were found most frequently, and $\alpha_1-2$ and $\alpha_1-3$ least frequently.

The electrophoretic mobility of the 11 $\alpha_2$ globulins extended from the mid-albumin region to the cathode side of the sample well. The most anodic of these, $\alpha_2-1$, was frequently obscured by the broad $\alpha_2-6$ precipitate, except at the anodic end. The $\alpha_2-2$ globulin, forming a long curvilinear precipitate, was displaced characteristically at its anodic end, and stained readily with protein- and carbohydrate-specific stains. The $\alpha_2-5$ globulin formed the only precipitate that was stained by the lipid-specific stain Oil Red O. It was stained very slightly by protein-specific stains. The lateral displacement of its precipitate from the diffusion center was very slight. The broadest precipitate in the $\alpha_2$ region was formed by $\alpha_2-6$. Depending on the duration of the incubation period and the sample volume, the apex sometimes extended into the antibody reservoir. The $\alpha_2-7$ globulin formed a long symmetrically curved precipitate with the apex located just anterior to the antigen well. It was usually obscured except at the ends by the albumin, $\alpha_2-6$, and $\beta_1-1$ precipitates. Two other precipitates, $\alpha_2-8$ and $\alpha_2-9$, were occasionally found directly over the antigen well. Both had very slight lateral displacement and usually fused...
posteriorly. The anodic ends of both $\alpha_2$-10 and $\alpha_2$-11 were usually completely obscured by the larger $\alpha_2$ globulin precipitates. The mobility and the geometric characteristics of their precipitates could not be determined.

The mobility of the six $\beta_i$ globulins extended from the mid-$\alpha_2$ to the mid-$\beta_5$ globulin regions. The most prominent $\beta_1$ globulin, and the one with the greatest lateral displacement, was $\beta_2$. The $\beta_2$-4 precipitate exhibited the greatest displacement variation; its length was frequently reduced by nearly one-half as it turned sharply downward past the cathodic end of the $\alpha_2$-6 precipitate. The $\beta_2$-5 globulin produced a nearly straight faintly visible precipitate with less lateral displacement than any other $\beta$ globulin. Only the curved end of the $\beta_2$-6 precipitate was usually visible; the remainder was covered by the $\beta_2$-1 precipitate.

Five $\beta_2$ globulin antigens were found, the most prominent of which was $\beta_2$. It formed a curvilinear precipitate that was markedly thickened and displaced at its cathodic end. The four other $\beta_2$ globulins were generally present, but were partially obscured by either the $\beta_2$-1 or $\gamma$ globulin precipitates. The $\beta_2$-5 precipitate usually appeared as a spur in the posterior part of the $\gamma$ globulin line.

The $\gamma$ globulin precipitate extended from the sample well to the most cathodic part of the immunoelectrophoretogram. Its precipitate was thickened and displaced at the cathodic end.

**Serum protein changes during tuberculosis.** Representative densitometric recordings of cellulose acetate membranes after electrophoresis of sera collected at various times after inoculation are shown in Fig. 1. No consistent differences were detected in the protein distribution between sera collected prior to and 7 days after inoculation. There was a marked increase in the $\alpha_0$ globulin content of the sera from three of the four guinea pigs bled after 14 days. The $\alpha_0$ globulins were substantially increased in the sera from all but one of the remaining animals bled at the time intervals indicated.

Immunoelectrophoresis revealed no consistent differences between sera collected prior to and 7 days after inoculation (Fig. 3).

Sera from two of the three guinea pigs bled 14 days after inoculation contained an antigenic $\alpha_2$ globulin which was not detected in normal sera. This antigen has been tentatively named $\alpha_2$-T. Its precipitate formed a dense, nearly symmetrical arc immediately below and nearly parallel to the concave surface of the $\alpha_2$-6 precipitate. It was stained by protein and carbohydrate stains but not by Oil Red O. The lateral displacement of its precipitate from the diffusion center was similar to that of albumin, $\alpha_1$-6, and $\beta_1$-1.

Only two ($\alpha_2$-4 and $\alpha_2$-5) of the six normal $\alpha_1$ globulins were found in the sera from three guinea pigs bled 21 days after infection. Comparison of the precipitate density between normal and post-inoculation sera from these same guinea pigs suggested that $\alpha_2$-4 was present in approximately normal amounts, whereas $\alpha_2$-5 was increased in all three sera. The $\alpha_{21}$-T antigen was found in all three sera. There was a depletion of the most cathodic constituents of $\beta_2$-4 which shortened the posterior aspect of its precipitate in all three serum immunoelectrophoretograms.

No consistent variation in the $\alpha_1$ globulins was observed in the immunoelectrophoretic patterns of sera collected at 28, 33, and 41 days after infection. The $\alpha_{12}$-T antigen was found in all of these sera. Composite immunoelectrophoretograms of normal sera and sera collected during the terminal stages of tuberculosis are shown in Fig. 2.

**Serum protein changes after sensitization with heat-killed M. bovis.** Cellulose acetate membrane electrophoresis and immunoelectrophoresis of sera from 12 adult male tuberculin-sensitive guinea pigs revealed no consistent differences when compared with their respective normal sera (Fig. 4). Hyper-$\gamma$-globulinaemia was found in one guinea pig bled after 15 days and in all four guinea pigs bled 21 days after sensitization.

**Discussion**

This study dealt with the serial changes in the serum proteins of guinea pigs infected with *M. bovis* and guinea pigs sensitized with heat-killed *M. bovis*.

Fulminating infection was produced in guinea pigs by inoculating 0.01 mg (wet weight) of *M. bovis* intraperitoneally. However, no changes in the electrophoretic or immunoelectrophoretic pattern of the serum proteins were found in the sera collected 1 week after infection.

The most striking and consistent changes occurred among the $\alpha_2$ globulins. Hyper-$\alpha$-globulinaemia was first detected 14 days after inoculation, and was found in all of the infected guinea pigs thereafter. Coincident with this change was the detection of an antigenic $\alpha_2$ globulin in the serum immunoelectrophoretograms of all but one of the infected guinea pigs. This antigen has been tentatively named $\alpha_2$-T. It has not been found in sera from 80 uninfected guinea pigs or in guinea pigs sensitized with heat-killed cells. However, $\alpha_2$-T is present in normal serum, since the antisera with which it was resolved were elicited by normal guinea pig sera. Therefore, it appears that the production of $\alpha_2$-T is greatly
stimulated between the 8th and 14th days after infection with *M. bovis*, and persists until death of the animal.

Some of the properties of $\alpha_2$-T can be inferred from its behavior during immunoelectrophoresis. It is a complete antigen which migrates with the $\alpha_2$ globulins during electrophoresis in agar-gel. Since it is stained by carbohydrate-specific but not lipid-specific stains, it appears to be a glycoprotein. It forms a dense symetrically curved precipitate with its apex near the edge of the antiserum basin. Therefore, it is probably not a macroglobulin, and it is present in relatively high concentration in the sera of tuberculous guinea pigs.

The simultaneous detection of $\alpha_2$-T and hyper-$\alpha$-globulinemia in the same sera suggests that the latter, a fairly consistent finding in advanced tuberculosis in many species, including man, may be caused by an increase in the serum concentration of $\alpha_2$-T. However, since both of these changes were detected by electrophoresis in different supporting media, it must be assumed that $\alpha_2$-T migrates as an $\alpha_2$ globulin in cellulose acetate as well as in agar-gel. That the mobility of certain proteins can be quite different in these two media is shown by the fact that the major guinea pig serum lipoproteins migrate as $\beta$ globulins on cellulose acetate and as $\alpha$ globulins in agar-gel. Therefore, it cannot be concluded that the eleva-
tion in the serum concentration of $\alpha_1$-T caused the hyper-$\alpha$-globulinemia. The temporal relationship that exists between these events, however, suggests this possibility.

Cellulose acetate electrophoresis and immunoelectrophoresis of sera from 12 guinea pigs that were sensitized to tuberculin with heat-killed *M. bovis* did not reveal either hyper-$\alpha$-globulinemia or $\alpha_1$-T. This suggests that both of these serum changes were dependent on the disease process and not on the development of delayed tuberculin hypersensitivity.

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LITERATURE CITED