Studies on the Dd antigen-antibody system. I. The nature of antigen Dd and its antibodies

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Summary. The nature of antigen Dd, an antigen present in the extracts of human dandruff which precipitates human sera selectively, and antibodies reacting with it are reported.

Antigen Dd, a component of certain human dandruff specimens, was first detected by Shrivastava[3]. The antigen precipitates human sera selectively and the frequency of reactors varies widely in different populations[3-5]. This paper reports our observations on some characteristics of antigen Dd and the antibodies reacting with it.

Materials and methods. Human dandruff samples were collected from several individuals and screened for presence of antigen Dd. All samples having the antigen were pooled and then processed according to Shrivastava[3] to obtain an extract containing antigen Dd.

The antigen-antibody reactions were monitored by immunoelectroosmophoresis (IEOP) in agarose gel (Koch-Light) following Rose and Bigazzi[6]. The IEOP plates, subsequent washing and drying, were stained with amido black 10B and in some cases with Sudan IV.

In an effort to see whether or not antigen Dd was present in human serum, we tested 50 randomly chosen fresh sera of Jat Sikh origin against an antigen Dd-reactor serum, both by IEOP and immunodiffusion. In addition, we tested 50 random cord sera of largely Jat Sikh origin to see if antigen Dd reactivity could be detected in them. We used also a commercial preparation of horse anti-human immune serum (Behringwerke) and an anti-human immune serum raised in goat, for our absorption experiments designed to see if antibodies against antigen Dd were present in these immune sera.

The electrophoretic position of antigen Dd was assessed by comparing the precipitation band formed in IEOP, as a result of reaction between antigen Dd and a reactor serum, with those formed by purified preparations of human serum albumin (Sigma) and human serum transferrin (Sigma) when reacted against anti-human immune serum.

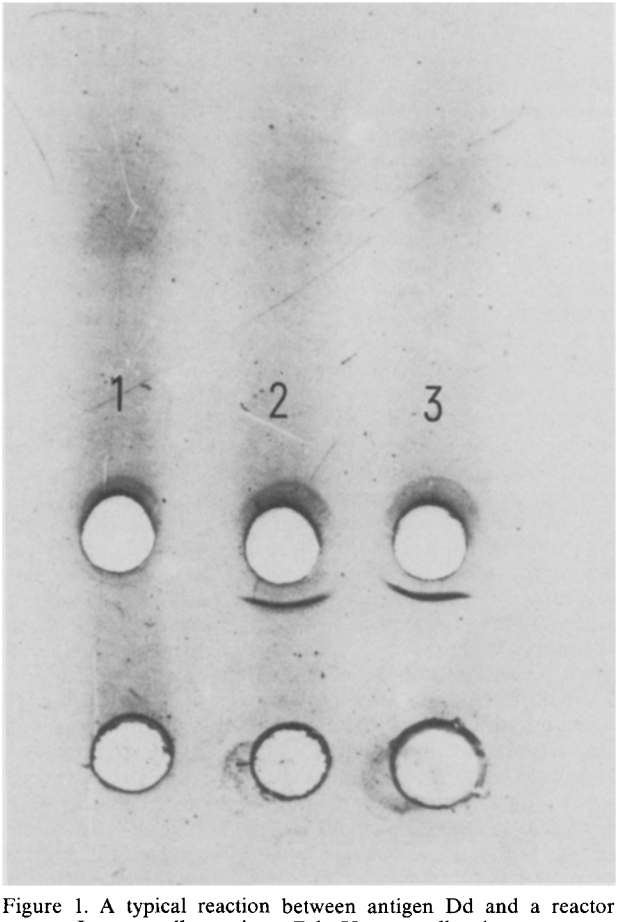

Figure 1. A typical reaction between antigen Dd and a reactor serum. Lower wells, antigen Dd. Upper wells; 1, non-reactor serum; 2 and 3, reactor sera. The anode is at the top.

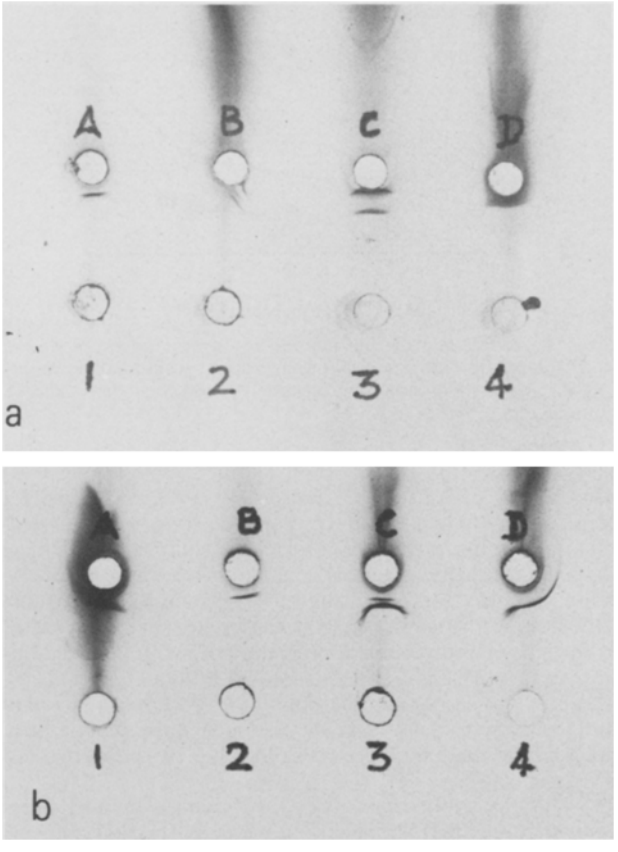

Figure 2. *a* Horse anti-human serum lacks antibodies against antigen Dd and antigen Dd has a mobility faster than transferrin and slower than albumin. Lower wells: 1 and 2, antigen Dd.; 3, human serum transferrin; 4, human serum albumin. Upper wells: A, antigen Dd-reactor serum; B, C and D, horse anti-human immune serum. IEOP in agarose gel. The anode is at the top.

b Antibody present in reactor serum is different from antibodies that react with horse anti-human immune serum. Lower wells: 1 and 4, anti-human immune serum diluted 1:2 with normal saline; 2, antigen Dd diluted 2:1 with normal saline; 3, antigen Dd absorbed 2:1 with anti-human immune serum. Upper wells: A, non-reactor serum, B, C and D, reactor sera. IEOP in agarose gel. The anode is at the top.

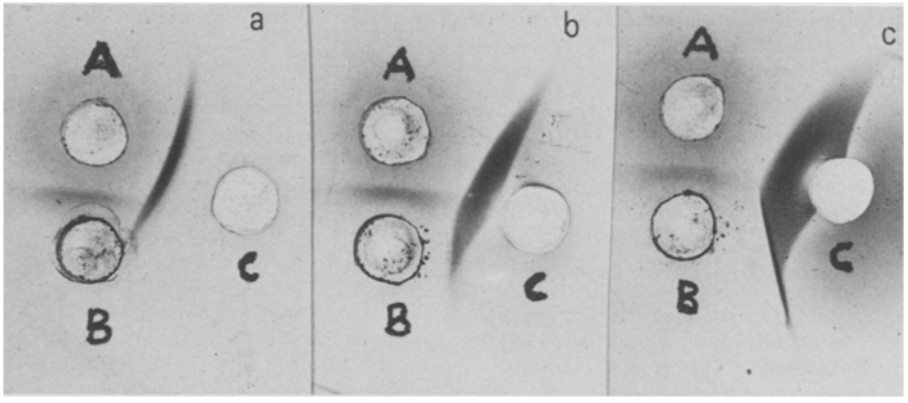

Figure 3. *a* The antibody against antigen Dd is not of IgM (μ-chain) type. A, reactor serum; B, antigen Dd; C, anti-IgM (μ-chain) immune serum. Note also absence of precipitin band between B and C. Immunodiffusion in agarose gel. *b* Antibody against antigen Dd is not of IgA (α-chain) type. A, reactor serum; B, antigen Dd; C, anti-IgA (α-chain) immune serum. Note also presence of precipitin band between B and C. Immunodiffusion in agarose gel. *c* Antibody against antigen Dd is not of IgG (γ-chain) type. A, reactor serum; B, antigen Dd; C, anti-IgG (γ-chain) immune serum. Note also presence of precipitin band between B and C. Immunodiffusion in agarose gel.

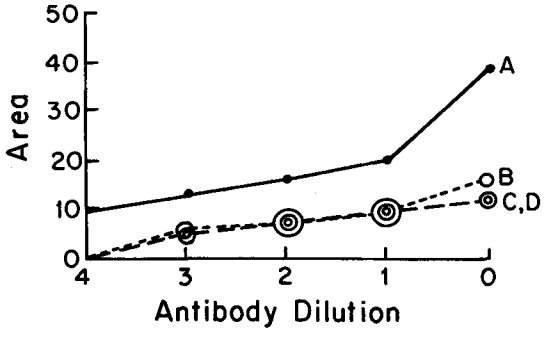

Figure 4. Results of radial immunodiffusion experiment, plotted to show quantitative differences among strong, weak and non-reactors for antigen Dd. A, strong reactor; B, weak reactor; C and D, non-reactors.

The nature of antibodies, present in antigen Dd-reactor serum, was studied by subjecting the serum to heat treatment according to Brzecka and Gajos[7] and also by testing the serum directly against specific anti-human immunoglobulin immune sera (Behringwerke) by immunodiffusion. The immune sera we used in these experiments were of rabbit origin and included anti-human IgM (μ-chain), anti-human IgA (α-chain) and anti-human IgG (γ-chain).

The effect of storage on the ability of a serum to react with antigen Dd was observed by testing against the antigen, reactor sera that had been stored at −20 °C for the last 3 years.

To study possible quantitative differences in antibodies against antigen Dd, present in various reactor sera, we made use of the technique of radial immunodiffusion as described by Mancini et al.[8].

Results and discussion. Figure 1 shows the type of precipitate that results when antigen Dd is tested against a serum having antibodies against it. The precipitates are always unequivocal and present no difficulty in identifying the reactor sera. It is interesting that while circulating antibodies against antigen Dd occur fairly frequently in human sera, we have failed to detect the antigen itself either in adult or in cord serum. Its absence from human serum was detected also by absorption experiments in which we had used anti-human immune sera of horse and goat origin (fig. 2). It would seem likely, therefore, that the antigenic stimulus responsible for the formation of antibodies against antigen Dd may be an indirect one.

Among the protein constituents of human dandruff extracts Berrens[9] has noted the presence of γ-globulin, α_2-glycoproteins and albumin. We have found these γ-globulins to be of the IgG and IgA types. The dandruff specimens we examined did not contain IgM (fig. 3). Antigen Dd appears to be a protein other than the ones just mentioned. In IEOP experiments it forms a band slightly cathodic to that of albumin but quite ahead of that formed by transferrin; this suggests a mobility of α_1-globulins or post-albumins for this protein. It is not stained by lipid dyes and, therefore, is not a lipoprotein. The exact nature of antigen Dd, however, remains to be elucidated. We have compared it with Australia antigen and have found no similarity between the two. The antigen retains its ability to form precipitate with reactor serum even when heated to 80 °C for 15 min.

The persistent nature of antigen Dd and its antibodies, the high frequency of reactors in some populations and a total lack of them in others[10], are factors which certainly do not rule out that antigen Dd may not be of extra-human origin. If the latter possibility is true, then the antigen could be a modified or degraded human protein in which the otherwise inactive or inaccessible sites have been rendered accessible or activated by the passage of the protein from its site of synthesis to the outermost epithelial layers. That such tissue components might become antigenic this way has been pointed out also by Gajdusek[11].

The results of our efforts to identify the antibodies reacting with antigen Dd have so far been inconclusive. All we can say at the moment is that these are neither of the IgM (μ-chain) type nor of the IgA (α-chain) and IgG (γ-chain) types (fig. 3) but can be of any other chain of these immunoglobulins, the antisera to which were not available. We have not been able to demonstrate them in cord serum. Also, these antibodies seem to be absent from the sera of such animals as sheep, goat, rabbit and fowl[12].

Our experiments have shown that prolonged storage at low temperatures does not affect the ability of a serum to react with antigen Dd. Reactor sera that had been stored at −20 °C still gave excellent results when tested against antigen Dd after 3 years. We have found also that there may be quantitative differences among reactor sera in regard to their antibody content. These observations are supported by results of our radial immunodiffusion experiments (fig. 4) with reactor and non-reactor sera. This

suggests that antibodies against antigen Dd may be universally present in man and what the latter detects might only be their raised amounts. Should it indeed be so then it would be interesting to seek an explanation for it.

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Endotoxin-like activity in *Pseudomonas paucimobilis* (group IIK biotype 1) and *Flavobacterium multivorum* (group IIK biotype 2)

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Summary. 7 strains of *Pseudomonas paucimobilis* and 7 strains of *Flavobacterium multivorum* (formerly groups IIK biotype 1 and biotype 2, respectively) showed endotoxin-like activity in vitro by the limulus amoebocyte lysate test.

Pseudomonas paucimobilis and *Flavobacterium multivorum* are typically yellow-pigmented, non-fermentative, gram-negative bacilli which were previously designated as groups IIK biotype 1 and biotype 2[1]. These organisms have often been isolated from human clinical specimens, hospital environments, and other sources[2,3]. However, these microbes have recently been reported to be causative agents of leg ulcer infection[4], of bacteremia[5,6] and of peritonitis[7]. Because of their roles as opportunistic pathogens, several investigations have been undertaken to better understand these bacteria. In one study, the cellular fatty acid composition of both groups was reported[8], and another study revealed that *P. paucimobilis* (biotype 1) was not immunologically related to *P. aeruginosa* or *P. cepacia* although complement-mediated bactericidal immunity was demonstrated[9]. Because of the need to further understand the pathogenesis of these organisms, especially since they are known to cause bacteremia[5,6], the present study was undertaken to test for the production of endotoxin in both groups of organisms.

Methods. 7 strains of *P. paucimobilis* and 7 strains of *F. multivorum* (kindly provided by Dr Robert Weaver, Centers for Disease Control, Atlanta, GA) were grown overnight at 35 °C on trypticase soy agar (BBL Microbiology Systems, Cockeysville, MD). The bacteria were harvested by washing the surface of each plate with 3 ml of sterile, pyrogen-free water. The cell suspensions were washed twice in the pyrogen-free water and then diluted to 50% transmission at 535 nm on a spectrophotometer. Bacterial suspensions were further diluted to yield $1:10^5$, $1:10^6$, and $1:10^7$ bacteria and were tested at each of these concentrations. Drying of aliquots of cells was done at 60 °C in an oven until the weights were constant.

Endotoxin detection was done by the limulus amoebocyte lysate test (Mallinckrodt, Inc., St. Louis, MO) using a microdilution procedure previously described[10]. Briefly, lysate was prepared with pyrogen-free water as recommended by the manufacturer, and equal volumes of lysate and bacterial suspensions were added in triplicate to microtiter plate wells, mixed, and incubated at 37 °C for 1 h. In order to determine the presence or absence of gelation, 0.01 ml of aqueous crystal violet was added to each well and then the lysate was viewed as previously described[10].

In addition, sensitivity of the lysate was confirmed using a standard reference endotoxin EC-2 (Mallinckrodt, Inc., St. Louis, MO) which yielded a minimum lysate sensitivity of 0.125 ng. EC-2 endotoxin was also run with each bacterial strain as a positive control.

Results and discussion. All 7 strains of both *P. paucimobilis* and *F. multivorum* yielded positive limulus tests at a concentration of $1:10^5$ bacteria per ml. 4 out of 7 *P. paucimobilis* strains exhibited positive endotoxin-like activity at $1:10^6$, and 5 of 7 *F. multivorum* strains yielded positive reactions at $1:10^6$ bacteria per ml. Thus, members of groups IIK biotype 1 and biotype 2 were highly reactive in in vitro gelation of lysate with the reference endotoxin. The sensitivity of the lysate test was 0.125 ng, and the present bacteria containing endotoxin-like substance is shown in the table.

Although only whole cells were tested, all strains of both genera were consistently positive in the lysate assay at a $1:10^5$ dilution. This suggests that these bacteria do possess some in vitro biological properties associated with endotoxin-like activity. These data are consistent with cellular fractionations by gas-liquid chromatography which detected hydroxy fatty acids in both genera. The difference of

Endotoxin-like activity of *Pseudomonas paucimobilis* and *Flavobacterium multivorum*

	P. paucimobilis	*F. multivorum*
Endpoint dilution*	10^5	10^5
Dry weight of bacteria, ng/ml	0.50	0.45
'Endotoxin' in bacteria, %	25	28

*Results expressed at the highest dilution of bacterial suspension at 50% transmission in which 100% of strains induced gelation.