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Immunoglobulin G Subclasses of Fluorescent Anti-*Treponema pallidum* Antibodies: Evidence for Sequential Development of Specific Anti-*T. pallidum* Immunoglobulin G Responses in Patients with Early Syphilis

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The development of immunoglobulin G (IgG) subclass-specific anti-*Treponema pallidum* antibodies during the course of syphilis in humans was studied with sera from 50 untreated male patients. The patients were divided into five diagnosis groups. In the fluorescent treponemal antibody test, which delineates the presence of cross-reacting antibodies, as well as specific antitreponema antibodies, IgG1, IgG2, and IgG3 subclass antibodies were already present during the seronegative primary stage. Specific antibodies, which were detected by the fluorescent treponemal antibody absorption test, were first present during the serotype-variable primary stage. These antibodies were almost exclusively of the IgG1 and IgG3 subclasses. In later stages, antibodies of other subclasses were detectable. Titration of IgG1 antitreponema antibodies in three electrophoretically different IgG fractions revealed an asymmetric distribution in these fractions during primary syphilis. The antibodies were largely confined to the most basic fraction during primary syphilis. A sudden change in the distribution was noted between the end of the primary stage and the secondary stage; an even distribution of IgG1 antitreponema antibodies existed in the late latent stage. These findings confirm and extend previous results from our laboratory. The development of antibodies detected by both tests is discussed in terms of a sequential stimulation of the immune system due to the presence of an extracellular layer covering the treponemas or, alternatively, in terms of a suppression of the immune response during early syphilis.

The development of immunity to syphilis is poorly understood. In patients in which the disease had reached latency (18) and in experimental animals approximately 3 months after the initial infection (30), resistance to intradermal challenge with pathogenic treponemas was observed. The degree of protection provided by infections for shorter periods correlates with the duration of infection (18, 30). This suggests that for the development of immunity, the disease has to follow its natural course for a rather long period.

The relative contributions of humoral and cellular immune mechanisms to the defense against treponemas in syphilis are unknown. The role of a humoral mechanism has been investigated by the transfer of an immune serum to normal rabbits. Several investigators (20, 24, 29) observed a delay in lesion development and less severity of the lesions with an absence of ulceration and reported a failure to demonstrate the presence of treponemas in the lesions. Protection against challenge during prolonged periods was obtained by a series of injections with immune sera (3, 37). However, in these studies, lesions appeared in at least some of the protected rabbits after the cessation of immune serum injections and protection against asymptomatic infection was not obtained (3). Titus and Weiser (28) provided evidence that the active entity was immunoglobulin G (IgG).

The slow development of host defense mechanisms on the one hand and the absence of positive results in certain in vivo and in vitro immunological tests (14, 16, 26, 27) on the other could complement each other and led to the hypothesis that the immune system is suppressed during early syphilis. However, it is not clear whether some of these findings are attributable to either a suppressed or a not yet sensitized immune system. We previously reported evidence for a slow development of IgG responses (33). In the present study, we extended these results by comparing the development of IgG subclass-specific anti-*Treponema pallidum* antibodies in the sera of patients with various stages of syphilis by using the fluorescent treponemal antibody (FTA) and FTA-absorption (FTA-ABS) tests. Furthermore, it was anticipated that a comparison of the development of nonspecific and specific antitreponema antibodies would provide further information on a possible suppression of the immune system with respect to IgG production during early syphilis.

MATERIALS AND METHODS

Sera. Serum samples were obtained from untreated male patients, 18 to 73 years old (average age, 37 years), and stored at −70°C until use. The clinical diagnosis of syphilis was made by the criteria described by Menke et al. (H. E. Menke, Ph.D. thesis, Erasmus University, Rotterdam, The Netherlands, 1975; N. H. L. de Jong, J. J. van der Sluis, J. van Dijk, and T. E. W. Feltkamp, Letter, Br. J. Vener. Dis. **54**:283, 1978) and was confirmed by positive dark-field examination or a positive *T. pallidum* immobilization reaction or both. The diagnosis of primary syphilis was differentiated by using four serological reactions (the Wasserman-Kolmer complement fixation reaction, the Reiter protein complement fixation reaction, the Venereal Disease Research Laboratory test, and the *T. pallidum* immobilization reaction). These reactions were performed as reported previously (33). Patients whose sera showed a negative reaction in all four tests were classified as seronegative (S 1−), patients whose sera were reactive in all four tests were classified as seropositive (S 1+), and patients whose sera

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were reactive in at least one of the tests (but not in all four) were classified as serotype variable (S 1±). Patients with secondary syphilis (S II) showed the typical papular or macular skin rash, and their sera were reactive in all four reactions. The incubation times (in weeks) of these stadia were as follows: S 1−, 3.0 ± 1.1; S 1±, 3.9 ± 1.1; S 1+, 5.9 ± 1.4; S II, 12.4 ± 4.3 (Menke, Ph.D. thesis). The patients with late latent syphilis (LL) had no clinical manifestations of infection, and their case histories showed no previous history of syphilis or syphilis treatment. In all cases, their sera had shown positive results in the *T. pallidum* immobilization reaction on one or more occasions, whereas the results of the cardiolipin reactions were negative or showed only a low antibody titer. Ten sera from each of the five diagnosis groups were investigated. Patients in whom concomitant disease was diagnosed were excluded from the study. Ten sera obtained from healthy blood donors were used as controls.

IgG fractions. Three IgG-containing fractions, differing in electrophoretic mobility, were prepared from five individual serum samples from each of the diagnosis groups, except the S 1− group, by DEAE-Sephadex ion-exchange chromatography. Isolation of the fractions and densitometric control of their charge differences were performed as described previously (33). Fraction 1 contained the most basic IgG molecules, and fraction 3 contained the most acidic. The protein concentrations in the fractions were estimated from the extinction at 280 nm, with human IgG as the standard. The protein content was adjusted to 1 mg/ml before use.

FTA slides. The following procedures were used for the preparation of FTA slides. The pathogenic Nichols strain of *T. pallidum* was used as the antigen. The organisms were extracted in Nelson medium by our standard procedure (34) from the testes of a rabbit which had been inoculated 1 week earlier. An equivalent amount (milliliters) of medium was added to the minced testicular tissue. The extraction was performed by shaking for 45 min under an atmosphere of 95% N_2–5% CO_2. After decantation, the supernatant was centrifuged at 800 × *g* for 10 min to remove particulate matter. Next, the treponemas were sedimented by further centrifugation at 40,000 × *g* for 15 min. The treponemas were washed once with phosphate-buffered saline (PBS) containing 0.01% NaN_3 (wt/vol) and stored in 2-ml aliquots at −70°C. FTA slides were prepared on Cooke microprint stock slides (Nutacon; CL 100; Cell-Line Associates, Inc., Newfield, N.J.) containing 10 spots per slide. The thawed treponemal suspension was adjusted to 30 treponemas per microscope field (380×) and applied to the spots with a wire loop. The slides were air dried, left overnight, and fixed in dry acetone for 10 min before storage at 4°C.

Antisera. Antisera against the four human IgG subclasses, raised in sheep, were obtained from the Central Laboratory of the Netherlands Red Cross Blood Transfusion Service, Amsterdam. According to the supplier, these antisera were specific for their subclass in the passive hemagglutination assays with erythrocytes coated with IgG of the various subclasses and in passive hemagglutination inhibition assays with IgG of the relevant subclass. The method of their production has been reported (7). The specificity of these antisera has been verified by others with different techniques (15, 17, 21), including fluorescence methods (31, 36). Fluorescein isothiocyanate-labeled rabbit anti-sheep IgG was obtained from the same supplier and used as the conjugate.

Absorptions. Preliminary experiments showed that the antisubclass antisera were reactive with *T. pallidum*. This reactivity was successfully removed by absorption with *T. pallidum* Nichols. A pellet of 10^8 treponemas was suspended in 0.2 ml of each of the antisubclass antisera. The mixture was incubated for 30 min at 4°C, and the treponemas were removed by centrifugation. To minimize nonspecific background staining of IgG2 subclass antiserum and IgG serum fractions, absorption with normal rabbit testes powder, prepared as described by Johnson et al. (13), was performed. Approximately 10 mg of testes powder was suspended in PBS and sedimented by centrifugation. The pellet was suspended in 1.0 ml of the appropriate dilution of the antiserum or serum fraction, and the mixture was incubated for 30 min at 4°C with occasional shaking. The testes powder was sedimented by centrifugation, and the supernatant was used in further experiments. The Reiter ultrasonic extract, which was used to absorb patient sera and serum fractions in the FTA-ABS test, was obtained from the Dutch National Institute of Health, Bilthoven, The Netherlands. It was prepared by sonifying 50 g (wet weight) of *Treponema phagedenis* biotype Reiter, suspended in 1 liter of PBS (pH 7.4), at 0°C for 10 min with a Sonifier (Branson Sonic Power Co., Danbury, Conn.) operated at 100 W. The final sonic extract was obtained from this crude mixture by centrifugation at 5,000 × *g* for 45 min. As a preservative, 1 ml of 40% formaldehyde was added per 100 ml of the final sonic extract.

Working dilutions. The antisubclass antisera were used at a dilution of 1:10 in combination with a 1:80 dilution of the conjugate. Although checkerboard titrations of the conjugate and the antisubclass antisera on FTA slides incubated with a 1:5 dilution of S II sera showed plateau levels at much higher dilutions (IgG1, 1:80; IgG2, 1:40; IgG3, 1:40; IgG4, 1:20; in combination with a 1:160 dilution of conjugate), lower dilutions were chosen to avoid false-negative results due to overdiluted antisera in combination with weak syphilitic sera. The controls (see below) performed on each occasion indicated that this introduced no false-positive results.

FTA procedure. Sera were tested in a 1:5 dilution with either PBS (FTA test) or an ultrasonic extract of *T. phagedenis* biotype Reiter (FTA-ABS). After a 30-min preincubation at room temperature, 20 μl of diluted serum was applied to the spots, and the slides were incubated for 30 min in a moist chamber at room temperature. The slides were rinsed and then washed with PBS containing 2% Tween 80 for 30 min with continuous stirring. The washing fluid was changed twice during this period. After the slides were air dried, 20 μl of diluted antisubclass antiserum was applied to the spots. After incubation, washing, and drying as described above, 20 μl of diluted conjugate was applied, and the incubation, washing, and drying procedure was repeated. Next, 2 drops of mounting medium, consisting of 1 part PBS and 9 parts glycerol, were applied to the slides. The slides were covered with a cover glass and sealed with nail polish.

Serum fractions containing 1 mg of IgG per ml were tested by the FTA-ABS procedure in a double dilution series. The titer of each fraction was recorded as the reciprocal of the highest dilution which gave a 1+ positive reaction. The mean titer and standard deviation were calculated from $\log_2$ values. For graphical purposes, these values were converted to dilutions. The experiments were arranged in such a way that only sera or serum fractions from one diagnosis group and one type of antisubclass antiserum were applied to a slide.

Controls. The following controls were used. The positive control comprised antigen incubated with S II serum, anti-IgG subclass antiserum, and conjugate. The negative controls were as follows: (i) antigen incubated with conjugate,

TABLE 1. Number of patients showing positive results for each of the four IgG subclasses in the FTA and FTA-ABS tests

IgG subclass	No. of patients showing positive results for indicated stage[a] in:									
	FTA test					FTA-ABS test				
	S 1−	S 1±	S 1+	S II	LL	S 1−	S 1±	S 1+	S II	LL
IgG1	3	10	10	10	10	0	10	10	10	10
IgG2	4	10	10	10	10	0	1	10	10	3
IgG3	2	10	10	10	10	0	9	10	10	6
IgG4	0	1	7	10	9	0	0	0	1	0

[a] Ten patients were tested per stage.

(ii) antigen incubated with S II serum or the appropriate IgG fraction and conjugate, (iii) antigen incubated with antisubclass antiserum and conjugate, and (iv) antigen incubated with control serum, anti-IgG subclass antiserum, and conjugate. In these controls, S II serum and the control serum were diluted 1:5 in either PBS or Reiter ultrasonic extract. The negative controls gave consistently negative results.

Microscopic examination. Slides were examined with a Leitz Orthoplan binocular microscope equipped with an epiilluminator. The light source was a xenon XBO 75 lamp; the filter combination was a BG 38 filter (4 mm) and filterblock K (Leitz) containing the excitation filters 2× KP 490 and GG 475 (2 mm), the dichroic mirror TK 510, and the barrier filter K 515. The microscope was equipped with a dark-field illumination (condenser D 1.20) objective (95×) with an adaptable numerical aperture and 4× oculars. The slides were observed under alternating dark-field and UV illumination. The results with UV light were scored as follows: 4+, very strong fluorescence; 3+, strong fluorescence; 2+, definite fluorescence; 1+, weak fluorescence; ±, treponemas were recognized without any specific fluorescence; and −, neither specific fluorescence nor treponemas were recognized, but treponemas could be recognized under dark-field illumination. The results were finally recorded as either positive or negative. The ± and − reactions were considered negative.

RESULTS

The results of the FTA test are shown in Table 1. It can be seen that the IgG1, IgG2, and IgG3 subclass antibodies appeared simultaneously. Within these three subclasses, a minority of the S 1− stage sera showed positive reactions. In the S 1± stage, all sera showed strong positive reactions, which persisted during the more progressive stages. The first antibodies that were detectable with anti-IgG4 antiserum were observed in the S 1± stage for one serum. The number of positive serum samples with this antiserum increased to 7 for the S 1+ stage patients and to 10 for the S II stage patients. In the LL stage, nine patients showed a positive IgG4 reaction. All 10 donor serum samples were negative in both the FTA and FTA-ABS tests.

The results of the FTA-ABS test were as follows (Table 1). All sera from S 1− stage patients showed a negative reaction with the four subclass antisera. In the S 1± stage, all 10 patients showed a positive reaction with anti-IgG1 and 9 showed a positive reaction with anti-IgG3. Only one patient in this group was positive with anti-IgG2. For the S 1+ stage patients, all sera showed positive reactions with anti-IgG1, -IgG2, and -IgG3 antisera. This remained during the S II stage. In the LL stage, strong positive reactions were found for all 10 sera with anti-IgG1. In this stage, six serum samples gave positive reactions for IgG3 and three gave positive reactions for IgG2. In the FTA-ABS reaction, no positive results were obtained with anti-IgG4 antiserum, except for one S II stage serum.

The results of the FTA-ABS test showed a successive involvement of the various IgG subclasses in the antitreponema response, which followed the sequence IgG1, IgG3, IgG2, and finally IgG4 in one S II stage patient. This is compatible with a shift of antitreponema antibodies from the basic type of heterogeneous IgG to a more acidic type during early syphilis. Accordingly, IgG fractions of different electrical charge were isolated and titrated for their IgG1 antitreponema antibodies. The initial IgG content of the various fractions was adjusted to 1 mg/ml before titration. The results of these titrations are shown in Fig. 1. It can be seen that in the S 1± and S 1+ stages basic fraction 1 contained most of the antitreponema activity. In the S 1+ stage, antibodies became detectable in fractions 2 and 3, with fraction 2 containing a slightly higher level of antibody activity than fraction 3. However, in the S II stage, high titers were found in all three fractions. In fraction 1 the mean titer showed a 3.5-fold increase as compared with the titer of this fraction in the S 1+ stage; the increase in fraction 2 and fraction 3 was 14-fold. In the LL stage, the mean titers of fractions 1, 2, and 3 were 1:10.6, 1:3.5, and 1:5.3, respectively. This shows that eventually an even distribution of antitreponema activity developed in the three electrophoretically different IgG fractions.

DISCUSSION

A comparison of the results of the FTA and FTA-ABS tests shows that the demonstration of antibodies of the four IgG subclasses in the S 1− and S 1± stage patients was different in the two tests. In the S 1− stage, some patients showed positive results for IgG1, IgG2, and IgG3 in the FTA test; in this stage all patients showed negative results for the four IgG subclasses in the FTA-ABS test. In the S 1± stage, all patients showed positive reactions for IgG1, IgG2, and IgG3 in the FTA test; in the FTA-ABS test, positive reactions were confined to the IgG1 and IgG3 subclasses, with the exception of one patient, who was positive for the IgG2 subclass. These results provide additional support for the specificity of the subclass antisera. They exclude cross-reactivity between the IgG4 subclass and the other subclasses (FTA test, S 1± stage), between IgG2 and IgG1 or IgG3 (FTA-ABS test, S 1± stage), and between IgG1 and IgG2 or IgG3 (FTA-ABS test, LL stage). This confirms the reported specificity (15, 17, 21, 31, 36) of the subclass antisera from the supplier. Based on this specificity, we conclude that from the end of the primary stage onward, antitreponema antibodies were found in all four IgG subclasses when the FTA test was used and in the IgG1, IgG2,

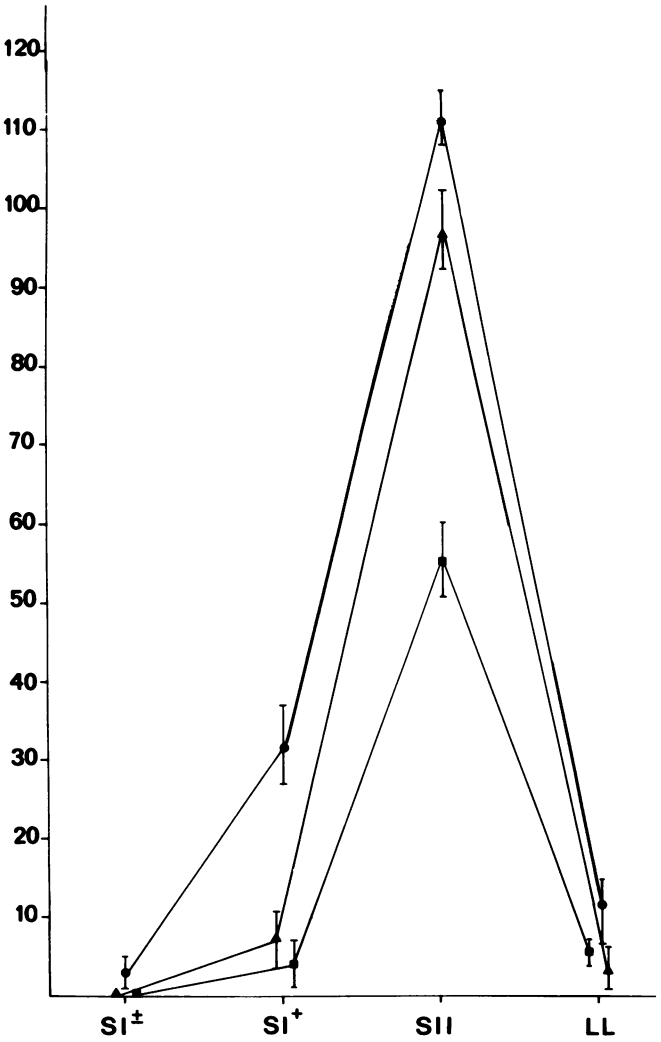

FIG. 1. Titration in FTA-ABS test of IgG1 subclass antitreponema antibodies in three IgG-containing fractions differing in electrophoretic mobility. The initial IgG content was 1 mg/ml. Symbols: ●, fraction 1; ▲, fraction 2; ■, fraction 3. The stages of human syphilis are shown on the abscissa; titers, as reciprocals of the highest dilutions giving a 1+ positive reaction, are shown on the ordinate.

and IgG3 subclasses when the FTA-ABS test was used. These results contradict those of Puritz et al. (22) who observed a restriction of antitreponema antibodies to the IgG1 subclass in patients with primary and secondary syphilis.

The complicated structure of the treponemas makes it difficult to compare the results for antitreponema antibodies with the results for subclass antibody formation to better defined antigens. The relatively few published studies of this kind show that, in humans, IgG antibodies to protein antigens (i.e., thyroglobulin [11], diphtheria toxoid [25], and tetanus toxoid [31]) are formed in the four IgG subclasses in quantities that parallel the relative amounts of the subclasses in normal serum. With tetanus toxoid there were indications that IgG1 subclass antibodies were produced first (31), but no charge relationship was investigated. However, the nature of the antigen may elicit antibodies belonging to selective subclasses. Antibodies to polysaccharide antigens (8, 38) and to grass-pollen antigen during immunotherapy (32) were produced preferentially in the IgG2 and IgG4 subclasses, respectively. It is not known whether such selective mechanisms play a part in the formation of antitreponema antibodies.

The simultaneous appearance of specific antitreponema antibodies of the IgG1 and IgG3 subclasses followed by the appearance of IgG2 subclass antibodies in syphilis patients resembles the sequence in which the IgG subclasses equilibrate at the basic end after isoelectrofocusing of pooled human IgG (12). IgG1 extends to a higher isoelectric point than does IgG3, which in turn extends to a higher isoelectric point than does IgG2. Control experiments showed that the absorption procedure efficiently removed antibodies that were reactive with *T. phagedenis* biotype Reiter from the donor sera as well as high-titered syphilitic patient sera. The FTA results demonstrate that the antibody conjugate system used readily delineated the presence of antitreponema antibodies of at least the IgG1, IgG2, and IgG3 subclasses. The present results therefore confirm our previous findings that an antitreponema IgG response starts with basic components of heterogeneous IgG. This results in an uneven distribution of antitreponema IgG antibodies during primary syphilis.

Because IgG1 is present in all heterogeneous IgG, the results with whole sera do not allow for a definite conclusion concerning a shift within the IgG1 subclass. An increase in the IgG1 antibodies could be due to an increase in antibodies already present at early infection or, alternatively, to a spread of IgG1 antibodies to the acidic side of IgG heterogeneity. The titration of the IgG fractions of different relative mobilities showed that antibodies are to a large extent confined to basic fraction 1 during the S 1± and S 1+ stages. In the secondary stage, all three fractions showed high titers, indicating that IgG1 subclass antibodies are subject to a similar shift through IgG heterogeneity. However, during the period between the S 1+ and S II stages a sudden change in the production of IgG1 antitreponema antibodies occurred. In the latter stage, high titers of antitreponema antibodies were present in all three fractions. This suggests that as late as the S II stage complete IgG heterogeneity is involved in antitreponema antibody production. This raises the question of whether the extension to more acidic antibodies involves previously uninvolved antigens. This seems likely because several investigators (2, 9, 19, 35) showed that with the *T. pallidum* polypeptides, electrophoretically separated on sodium dodecyl sulfate-polyacrylamide gels, almost all bands react on blots with IgG antibodies in sera from patients with secondary syphilis. However, only a limited number of bands react with IgG antibodies in sera from patients with primary syphilis. The results are similar for IgM antibodies (9, 19, 35).

This evidence indicates that during primary syphilis, specific IgG antibodies of limited heterogeneity arise against a limited number of treponemal components. These limitations are overcome during the secondary stage. This points to a slow sensitization of the immune system to some of the treponemal antigens. An explanation, which seems the most likely one, is that until the S II stage, an optimally functioning humoral immune system is not stimulated by the relevant antigens. This could be due to the inaccessibility of these antigens, either because they are located inside the treponemas or because they are covered by a protective layer surrounding the treponemas or both. The presence of such a layer was first hypothesized by Christiansen (4). Host serum proteins (1), as well as mucopolysaccharides, have been implicated (5, 39). Evidence that a layer acts as a barrier inhibiting the reaction of antibodies with treponemal

antigens has been obtained from serological tests. No antigen-antibody reactions occurred with freshly harvested treponemas; positive results were obtained only after a period of in vitro aging (10). This has been attributed to the loss of a protective layer.

A second explanation for the observed limited IgG response is that cellular processes underlying the production of IgG could be suppressed as a consequence of a more general suppression of cell-mediated immunity during syphilis. There is experimental evidence from in vitro lymphocyte stimulation experiments both supporting and contradicting such a suppression in patients with syphilis. Antigenic stimulation with whole *T. pallidum* Nichols cells showed a gradually increasing response of lymphocytes isolated from peripheral blood of patients with sequential progressive stages of syphilis (6). This favors the hypothesis of a gradually increasing sensitization to treponemal antigens and contradicts the immunosuppression hypothesis. The evidence favoring suppression stems from the diminished in vitro mitogenic stimulation of peripheral blood lymphocytes from patients. This suppression is maximal during the secondary stage and is attributed to one or more serum factors (16, 27). Since we found that the limitations of the initial IgG response were overcome just before the secondary stage, it seems unlikely that IgG production is connected to this postulated immune suppression. This conclusion is supported by the IgG production early in infection, as delineated by the FTA test.

A second observation often cited to support immune suppression during early syphilis is the absence of positive skin reactions to treponemal antigens in patients with primary and secondary syphilis, in contrast to results for patients with later stages of syphilis (14, 26). Since IgG production, as well as the elicitation of delayed-type hypersensitivity reactions, requires T lymphocytes presensitized to the relevant antigens, the absence of a positive skin reaction early in infection could be related to the limited sensitization demonstrated here.

The basic IgG response, as delineated by the FTA-ABS procedure, is suggestive of a mechanism involving an inverse charge relationship early in the syphilitic infection (23). Negatively charged molecules, such as mucopolysaccharides, could play a role in this mechanism by modifying the electrical charge of treponemal antigens. In this manner, antigens with a net negative carrier component could be provided, which in turn could give rise to the IgG response in the basic region.

LITERATURE CITED

1. **Alderete, J. F., and J. B. Baseman.** 1979. Surface-associated host proteins on virulent *Treponema pallidum*. Infect. Immun. **26**:1048–1056.
2. **Baker-Zander, S. A., E. W. Hook III, P. Bonin, H. Hunter Handsfield, and S. A. Lukehart.** 1985. Antigens of *Treponema pallidum* recognized by IgG and IgM antibodies during syphilis in humans. J. Infect. Dis. **151**:264–272.
3. **Bishop, N. H., and J. N. Miller.** 1976. Humoral immunity in experimental syphilis. I. The demonstration of resistance conferred by passive immunization. J. Immunol. **117**:191–196.
4. **Christiansen, S.** 1963. Hypothesis: protective layer covering pathogenic treponemata. Lancet **i**:423–425.
5. **Fitzgerald, T. J., R. C. Johnson, and E. T. Wolff.** 1978. Mucopolysaccharide material resulting from the interaction of *Treponema pallidum* (Nichols strain) with cultured mammalian cells. Infect. Immun. **22**:575–584.
6. **Friedman, P. S., and J. L. Turk.** 1975. A spectrum of lymphocyte responsiveness in human syphilis. Clin. Exp. Immunol. **21**:59–64.
7. **Goossen, P. C. M., S. van Beekhuizen, C. Droogh, and G. de Lange.** 1981. Preparation of antibodies against subclasses of human IgG. J. Immunol. Methods **40**:339–344.
8. **Hammarström, L., M. Granström, V. Oxelius, M. A. A. Persson, and C. I. E. Smith.** 1984. IgG subclass distribution of antibodies against *S. aureus* teichoic acid and α-toxin in normal and immunodeficient donors. Clin. Exp. Immunol. **55**:593–601.
9. **Hanff, P. A., T. E. Fehninger, J. N. Miller, and M. A. Lovett.** 1982. Humoral immune response in human syphilis to polypeptides of *Treponema pallidum*. J. Immunol. **129**:1287–1291.
10. **Hardy, P. H., and E. E. Nell.** 1957. Study of the antigenic structure of *Treponema pallidum* by specific agglutination. Am. J. Hyg. **66**:160–172.
11. **Hay, F. C., and G. Torrigiani.** 1974. The distribution of antithyroglobulin antibodies in the immunoglobulin G subclasses. Clin. Exp. Immunol. **16**:517–521.
12. **Howard, A., and G. Virella.** 1969. The separation of pooled human IgG into fractions by isoelectrofocusing and their electrophoretic and immunologic properties, p. 449–453. *In* H. Peeters (ed.), Protides of biological fluids. Pergamon Press, Ltd., Oxford.
13. **Johnson, G. D., E. J. Holborrow, and J. Dorling.** 1978. Immunofluorescence and immunoenzyme techniques, p. 15.13. *In* D. M. Weir (ed.), Handbook of experimental immunology. Blackwell Scientific Publications, Ltd., Oxford.
14. **Kiraly, K., and I. Racz.** 1960. Hautproben mit gereinigter *Treponema pallidum*-(TP) vaccine. Arch. Klin. Exp. Dermatol. **209**:583–591.
15. **Klein, F., F. Skvaril, R. Vermeeren, A. Vlug, and W. J. Duimel.** 1985. The quantification of human IgG subclasses in reference preparations. Clin. Chim. Acta **150**:119–127.
16. **Levene, G. M., J. L. Turk, D. J. M. Wright, and A. G. S. Grimble.** 1969. Reduced lymphocyte transformation due to a plasma factor in patients with active syphilis. Lancet **ii**:246–247.
17. **Magnusson, C. G. M., D. L. Delacroix, J. P. Vaerman, and P. L. Mason.** 1984. Typing of subclasses and light chains of human monoclonal immunoglobulins by particle counting immunoassay (PACIA). J. Immunol. Methods **69**:229–241.
18. **Magnusson, H. J., E. W. Thomas, S. Olansky, B. I. Kaplan, L. de Mello, and J. C. Cutler.** 1956. Inoculation of syphilis in human volunteers. Medicine (Baltimore) **35**:33–82.
19. **Moskophidis, M., and F. Müller.** 1984. Molecular analysis of immunoglobulins M and G immune response to protein antigens of *Treponema pallidum* in human syphilis. Infect. Immun. **43**:127–132.
20. **Perine, P. L., R. S. Weiser, and S. J. Klebanoff.** 1973. Immunity to syphilis. I. Passive transfer in rabbits with hyperimmune serum. Infect. Immun. **8**:787–790.
21. **Postoway, N., and G. Garratty.** 1983. Standardization of IgG subclass antiserums for use with sensitized red cells. Transfusion (Philadelphia) **23**:398–400.
22. **Puritz, E. M., J. A. Thompson, Jr., F. Dierberg, S. J. Kraus, and W. J. Yount.** 1975. IgG subclasses of fluorescent treponemal antibodies: correlation with complement fixation and clinical stage. Clin. Immunol. Immunopathol. **4**:352–361.
23. **Sela, M., and E. Mozes.** 1965. Dependence of the chemical nature of antibodies on the net electrical charge of antigens. Proc. Natl. Acad. Sci. USA **55**:445–452.
24. **Sepitjan, M., D. Salussola, and J. Thivolet.** 1973. Attempt to protect rabbits against experimental syphilis by passive immunization. Br. J. Vener. Dis. **49**:335–337.
25. **Spiegelberg, H. L.** 1974. Biological activities of immunoglobulins of different classes and subclasses. Adv. Immunol. **19**:259–294.
26. **Thivolet, J., A. Simeray, M. Rolland, and F. Challut.** 1953. Etude de l'intradermo-réaction aux suspensions de tréponèmes formolées (souche Nichols pathogène) chez les syphilitiques et les sujets normaux. Ann. Inst. Pasteur **85**:23–33.
27. **Thompson, J. J., R. J. Mangi, R. Lee, and J. M. Dwyer.** 1980. Immunoregulatory properties of serum from patients with different stages of syphilis. Br. J. Vener. Dis. **56**:210–217.

28. **Titus, R. G., and R. S. Weisser.** 1979. Experimental syphilis in the rabbits: passive transfer of immunity with immunoglobulin G from immune serum. J. Infect. Dis. **140**:904–913.
29. **Turner, T. B., P. H. Hardy, Jr., B. Newsman, and E. E. Nell.** 1973. Effects of passive immunization on experimental syphilis in the rabbit. Johns Hopkins Med. J. **133**:241–251.
30. **Turner, T. B., and D. H. Hollander.** 1957. Biology of the treponematoses. WHO Monogr. Ser. **35**:125–128.
31. **van der Giessen, M., and M. Groeneboer-Kempers.** 1976. The subclasses of human IgG antibodies against tetanus toxoid. Clin. Exp. Immunol. **25**:117–121.
32. **van der Giessen, M., W. L. Homan, G. van Kernebeek, R. C. Aalberse, and P. H. Dieges.** 1976. Subclass typing of IgG antibodies formed by grass pollen-allergic patients during immunotherapy. Int. Arch. Allergy Appl. Immunol. **50**:625–640.
33. **van der Sluis, J. J., and M. Boer.** 1980. Uneven distribution of antitreponema antibody activity in differing immunoglobulin G fractions from patients with early syphilis. Infect. Immun. **29**:837–841.
34. **van der Sluis, J. J., P. C. Onvlee, F. C. H. A. Kothe, V. D. Vuzevski, G. M. N. Aelbers, and H. E. Menke.** 1984. Transfusion syphilis, survival of *Treponema pallidum* in donor blood. I. Report of an orientating study. Vox Sang. **47**:197–204.
35. **van Eijk, R. V. W., and J. D. A. van Embden.** 1982. Molecular characterization of *Treponema pallidum* proteins responsible for the human immune response to syphilis. Antonie van Leeuwenhoek J. Microbiol. **48**:487–488.
36. **von dem Borne, A. E. G. K., F. M. Helmerhorst, E. F. van Leeuwen, H. G. Pegels, E. van Rieth, and C. P. Engelfriet.** 1980. Autoimmune thrombocytopenia: detection of platelet autoantibodies with the suspension immunofluorescence test. Br. J. Haematol. **45**:319–327.
37. **Weiser, R. S., D. Erickson, P. L. Perine, and N. N. Pearsall.** 1976. Immunity to syphilis: passive transfer in rabbits using serial doses of immune serum. Infect. Immun. **13**:1402–1407.
38. **Yount, W. J., M. M. Dorner, H. G. Kunkel, and A. E. Kabat.** 1968. Studies on human antibodies. VI. Selective variations in subgroup composition and genetic markers. J. Exp. Med. **127**:633–646.
39. **Zeigler, J. A., A. M. Jones, R. H. Jones, and K. M. Kubica.** 1976. Demonstration of extra-cellular material at the surface of pathogenic *Treponema pallidum* cells. Br. J. Vener. Dis. **52**:1–8.