Preference for basic IgG in early syphilis

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It is known that during a syphilitic infection the serum level of the three major immunoglobulin classes is raised (Delhanty and Catterall, 1969; Heitmann, 1972). There are antibodies present in the IgG, IgM, and IgA classes which can react with *T. pallidum*, as revealed by immunofluorescent studies in the FTA-ABS test (Király, Backhausz, Jobbágy, Lajos, and Kovats, 1968; Julian, Logan, and Norins, 1969). To our knowledge, however, no study has been reported relating these reactivities to the micro-heterogeneity of the immunoglobulins. In the preceding paper, we have presented evidence that syphilitic sera contain larger amounts of basic immunoglobulins of the IgG class than normal sera. It is not known whether this increase reflects a specificity of the immune response to infection with *T. pallidum* or is only the result of a hypergamma-globulinaemia (Delhanty and Catterall, 1969). In the present paper we attempt to answer this question by quantifying the total IgG content and the amount of basic IgG in syphilitic sera. The measurements are restricted to the seropositive primary, secondary, and early latent stages, as the secondary stage shows the largest elevations in IgG level. The other two stages might add further information, because they are closely related to the secondary stage.

As far as it was possible to follow the patients during a 6-week period of treatment, the effect of therapy on both total and basic IgG levels is also reported.

Material and methods

All reagents used were of analytical reagent grade.

Sera of syphilitic patients were used either at once or after storage at -70°C . for varying periods of time. In all cases the diagnosis was confirmed by a positive dark-field examination and/or a positive *Treponema pallidum* immobilization reaction. The 27 patients ranged in age from 19 to 67 years (mean 29). Nine were women. From the case histories it was highly probable that the duration of infection was shortest in patients with seropositive primary and longest in those with early latent syphilis. During the treatment period of 6 weeks, 31.2 mega units of penicillin were given by intramuscular injection.

The 25 members of the control group, selected from Army volunteers, appeared healthy on clinical examination and had normal values on general blood and urine examination. The group consisted of sixteen men and nine women, ranging in age from 19 to 48 years (mean 30). Serum IgG levels (referred to as total IgG level) were determined by the method of Mancini, Carbonara, and Heremans (1965) on Partigen Immunodiffusion Plates (Behringwerke Chemie, Marburg/Lahn, Germany). These measurements were done in duplicate on different plates. In every plate three standards were used (Nordic, Tilburg).

Total protein content of the sera was measured by the biuret reaction, using Lab-Trol (Dade, Miami, Florida, U.S.A.) as a standard. Paper electrophoresis of serum was performed in barbital-barbital sodium buffer, $\text{pH} = 8.6$, $\mu = 0.1$. After the strips had been stained in azocarmine B, they were cut in the standard protein regions, and eluted in 0.1 per cent. NaOH. From the ratio of the spectrophotometric readings of these solutions at 532 nm. and the total protein concentration, the protein content of the regions was calculated. Agar electrophoresis was performed on glass slides measuring 7.6×2.6 cm. 3 ml. of a warm 0.9 per cent. agar solution (Noble Agar, Difco) in barbital-barbital sodium buffer, $\text{pH} = 8.6$, $\mu = 0.05$, was applied to the slides. The next day samples of 5 μl . of two sera were applied to the agar layer on a slide by an application apparatus (LKB, Stockholm, Sweden).

Electrophoresis in the apparatus described by Wieme (1965) was performed for 28 min. at 15°C . The proteins on the slides were fixed by the method of van Vreedendaal (1967). The slides were stained in Amidoblack 10B according to the method of Uriel and Grabar (1956) and de-stained in five successive washes in 2 per cent. acetic acid solution. The slides were scanned with a densitometric attachment to a Vitatron spectrophotometer at 532 nm. by the method of Wieme (1965). Double diffusion experiments were done by the method of Ouchterlony (1958).

Statistical analysis of the distribution of total IgG and basic IgG levels was done by the non-parametric test of Wilcoxon.

Calculation of the amount of basic IgG

The distance from the albumin peak to the β globulin peak was measured on the pattern obtained after scanning. From this value the distance from the β globulin peak to the point of zero mobility was calculated by multiplying

by 48/52. This value, as determined by one of us (H.N.) for the standard conditions described, agrees well with the values obtained by Wieme (1965) and Uriel (1964) (*e.g.* 47/53 and 51/49) under slightly different conditions.

From this point one tenth of the total length of the separation pattern was arbitrarily defined as containing the basic IgG. The amount of protein in this area was calculated in the following manner: the globulin content of the serum (total protein minus albumin (paper)) was divided by the total number of integrations in the α_1 , α_2 , β , and γ region. This quotient was multiplied by the number of integrations found in the tenth part of the total separation pattern. This gives the concentration of basic IgG in grams per litre.

Results

The mean values of total IgG and basic IgG for the control group and the three syphilis groups are given in the Table. Using a level of significance of 0.01, statistical analysis gives the following results. Mean total IgG levels in untreated patients in the seropositive primary, secondary, and early latent stages differ significantly from the mean total IgG level in the control group. There is also a significant difference between the seropositive primary and secondary stages, but not between the secondary and early latent stages. The mean basic IgG levels in untreated patients in the three syphilis groups also differ significantly from that in the control group. There is a significant difference in basic IgG level between the seropositive primary stage and the secondary stage and also between the secondary stage and the early latent stage.

Penicillin treatment resulted in a statistically significant decrease in both the total IgG level and the basic IgG level in the secondary stage only.

Total IgG and basic IgG levels in treated patients in the secondary and early latent stages do not differ significantly from these levels in the control group. In order to compare the amounts of total IgG and of basic IgG with each other and to avoid direct comparison of values obtained by different methods, the total IgG level and basic IgG level of each patient

was expressed as a percentage of the mean values of their respective counterparts in the control group. In Fig. 1 a graphic representation of these percentages is given. The dotted line, drawn at an angle of 45° , would indicate an equivalent percentage change in both parameters. It can be seen that, in all but one of the eleven patients in the secondary stage, the ratio of % basic IgG to % total IgG lies above this line. This is also the case in four out of nine patients in the early latent stage and in four out of seven patients in the seropositive primary stage.

Fig. 2 shows the effect of therapy on the proportional ratio of percentages of both IgG levels in patients in the secondary stage. To avoid an over-estimation of the slopes, which are obtained by expressing the decreases proportionally, these slopes are compared with the slope of a line which is calculated from a decrease twice as high in absolute values of total IgG as in basic IgG (dotted line in Fig. 2). In nine out of eleven patients in the secondary stage the slopes are equal to, or even greater than, the slope of the dotted line.

The three patients in the seropositive primary stage, who could be followed during treatment, showed a similar decrease in their ratio (of %'s) basic IgG to total IgG, to most of the patients in the secondary stage. These patients initially had a ratio which fell above the line of 45° in Fig. 1. The same applied to patients in the early latent stage. The six patients with the highest ratio showed similar changes during treatment to patients in the secondary stage.

Discussion

In the measurement of the amount of basic proteins in electrophorograms, the area containing these proteins was arbitrarily defined as a tenth of the total length of the separation pattern. It is established that in this part of the γ -region only IgG is present. Uriel (1964) found only IgG in the range from 0 to 0.28. In double diffusion experiments on punches from the tenth part of the electrophorograms we could not detect proteins other than IgG.

TABLE Mean values, range, and standard deviation of total and basic IgG serum levels in syphilitic patients and control group

Stage	Therapy	No. of patients	Total IgG			Basic IgG		
			g./l.	Range	S.D.	g./l.	Range	S.D.
Seropositive primary	No	7	14.1	11.3-20.3	3.0	2.6	0.9-5.3	1.9
	Yes	3	13.6	11.5-16.7	—	2.0	1.4-3.2	—
Secondary	No	11	16.6	9.1-25.0	4.5	3.9	2.0-6.7	1.6
	Yes	11	12.8	8.1-16.3	2.7	1.7	0.6-4.6	1.5
Early latent	No	9	14.6	9.8-21.1	4.2	2.3	0.2-4.5	1.6
	Yes	9	12.4	7.2-16.8	3.4	1.6	0.4-3.2	1.1
Control group	No	25	10.3	7.1-12.4	1.4	1.5	0.6-2.8	0.5

FIG. 1 Abcissa: Total IgG level of 27 syphilis patients, expressed as percentage of mean total IgG of control group

Ordinate: Basic IgG level of same 27 patients expressed as percentage of mean basic IgG level of control group

In the secondary stage, in all but one of the untreated patients, the increase in basic IgG was greater than that in total IgG. Considering that the increase in basic IgG is also, but to a lesser extent, reflected in the proportional total IgG increase, it follows that during this stage of the disease there is a strong stimulation of the synthesis of the proteins in the basic part of the profile of total IgG micro-heterogeneity. This makes it very likely that, at least within the IgG class, antibodies with high isoelectric points are generated as a result of infection with *T. pallidum*. This is supported by the results of the effect of therapy on both the IgG levels. As can be observed in Fig. 2, the decrease in basic IgG level can account for about 50 per cent. of the decrease in total IgG level. In the control group, the basic IgG constitutes,

FIG. 2 Effect of penicillin therapy on total IgG and basic IgG levels in eleven patients with secondary stage syphilis

Abcissa and ordinate as in Fig. 1.

Slope of dotted line calculated from decrease in total IgG twice as great as in basic IgG

as an estimate, only 15 per cent. of the total IgG. Thus, this small part of the total IgG accounts to a large extent for the decrease in total IgG level. As the therapeutic effect of penicillin on *T. pallidum* is well established, it seems obvious that the decrease in immunoglobulins with high isoelectric points after treatment is caused by a less strong antigenic stimulation.

The number of *T. pallida* present in the body is believed to increase gradually from the seronegative stage up to the secondary stage. Thereafter, the number of micro-organisms decreases gradually (Collart, Franceschini, and Durel, 1971). There is in untreated patients a significant difference, in both total IgG and basic IgG levels, between the seronegative primary and the secondary stages.

Between the secondary stage and the early latent stage no significant decrease in total IgG is found. However, basic IgG has decreased significantly. These facts suggest a closer relationship of the fluctuations in the number of treponemes with basic IgG levels than with total IgG levels.

The high ratio of % basic IgG to % total IgG, found in almost all patients in the secondary stage, makes it highly probable that the high ratios found in untreated patients with a diagnosis of seropositive primary or early latent syphilis, indicate a stage of the disease not far from the secondary stage. The increase in basic IgG after infection with *T. pallidum*, the sharp decline of its level after penicillin treatment, and its spontaneous decrease in the early latent stage suggest that the increase in basic IgG may be the result of preferential synthesis of high isoelectric point antibodies, which are directed against *T. pallidum*.

Additional work on this subject is in progress.

Summary

By differential IgG measurements of sera of 27 patients with early infectious syphilis, it was found that infection with *T. pallidum* results in a preferential synthesis of IgG immunoglobulins characterized by high isoelectric points. The decrease in total IgG level

observed after 6 weeks of treatment can to a large extent be accounted for by the decrease in basic IgG concentration. The relationship between the total and basic IgG levels and the number of *T. pallidum* present in the body during the three early stages of the disease studied is discussed.

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