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B lymphocyte population and immunoglobulins in Indian kala-azar in response to chemotherapy

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Summary

The levels of immunoglobulin classes (IgG, IgM and IgA) along with B lymphocyte population size were estimated in 24 kala-azar (KA) patients and results were compared with those obtained from 30 controls. A marked increase in the level of IgG and to a lesser extent in that of IgM was noted in the sera of KA patients. But, no such difference could be noted in serum IgA level. Along with the increased levels of immunoglobulins the elevation of B lymphocyte population size was also observed. Follow-up studies during and after chemotherapy for the period of 8 months showed that clinical improvement in KA patients resulting from treatment had a positive correlation with the progressive decline in the B lymphocyte population and in the level of immunoglobulin though the latter, particularly IgG, took a longer time to return to normal.

Key words: kala-azar; immunoglobulin level; B lymphocyte population; anti-leishmanial treatment.

Introduction

Inordinate elevation in the level of gammaglobulin following increased production of plasma cells is a characteristic feature of kala-azar (KA) (Chaves and Ferri, 1966; Irunberry et al., 1968). Marked increase was noted in the serum level of both IgM and IgG classes of immunoglobulins (Chaves and Ferry, 1966; Irunberry et al., 1968; Ghose and Chowdhury, 1977; Aikat et al., 1979; Ghose et al., 1980), more so in IgG level (Manson-Bahr, 1971; Turk and Bryceson, 1971; Bray, 1972; Marsden, 1979; Ghose et al., 1980) during the active stage of the

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disease. It is also known that raised level gradually goes down to normal as the patients move towards cure following treatment (Mackelt, 1972).

But the pattern of waning of immunoglobulin's level is yet to be elucidated clearly. It is known that with the help of T lymphocytes (helper T cells), B lymphocytes lead to the production of plasma cells, which actively synthesize the immunoglobulins and consequently antibodies. High rise of immunoglobulin level and antibody titre in the sera of KA patients reasonably calls for information about B lymphocytic activity during the disease process. As the numerical strength of lymphocytes may reflect indirectly their probable activity, the quantitation of B lymphocytes might be used to investigate the relationship between the B lymphocyte population and the immunoglobulin level during various stages of the disease.

In the present study, the levels of immunoglobulins and B lymphocyte population size in KA patients were determined before treatment and sequentially for 8 months thereafter.

Materials and Methods

Patients, treatment and collection of sample

Twenty-four KA patients included in this study attended Bandipur Primary Health Centre, 24 Parganas, West Bengal, for treatment during the recent outbreak of this disease in the area. Diagnosis was made by careful clinical and parasitological examinations. The patients had temperature rising up to 40°C, enlarged spleen (2.5 to 10 cm below the costal margin), liver (1.5 to 6.0 cm below the costal margin) and loss of weight. Anaemia (4.0 to 7.8 g/dl haemoglobin) and leucopenia (1800 to 4200 leucocytes per mm³ of blood) were the characteristic features.

For treatment, patients received sodium antimony gluconate (SAG) (Gluconate Ltd., Calcutta, India) according to drug schedule (Table 1). As indicated by subsidence of temperature, restoration of blood values, e.g. correction of anaemia and leucopenia, regression of hepatosplenomegaly and weight gain, all of the patients became clinically cured within 2 months after receiving 2 courses of SAG. As of the patients clinically cured and remaining free from any clinical manifestation during the period of observation after treatment very few were available for re-examination of bone marrow, parasitological cure could not be established conclusively.

Table 1. Treatment schedule for kala-azar patients

Sodium antimony gluconate (SAG):

Available as injection obtained in 30 ml vial (100 mg/ml)

Test dose:

Children – 0.5 ml I.M.

Adult – 1.0 ml I.M.

Subsequently:

1 year of age – 1 ml I.M. daily × 12 days

1–3 years of age – 2 ml I.M. daily × 12 days

4–7 years of age – 3 ml I.M. daily × 12 days

8–14 years of age – 4 ml I.M. daily × 12 days

Adult – 5 ml I.M. daily × 12 days

The course was repeated after 2 weeks in each case.

Peripheral blood (heparinized and non-heparinized) was collected before treatment and then at monthly interval up to 6 months and finally at 8 months after commencement of treatment. Blood samples were also collected from 30 normal healthy individuals (controls) of the same endemic area. Serum was separated by the centrifugation of clotted blood. Heparinized blood was used for quantitative estimation of B lymphocyte population.

Immunoglobulin estimation

The quantitative estimation of immunoglobulins (IgG, IgM and IgA) was performed by radial immunodiffusion (Mancini et al., 1965) using a commercially available immunodiffusion Kit (Hoechst Pharmaceuticals Ltd., India). IgM and IgA were determined using undiluted sera. For IgG determination, patient's sera and control sera were diluted 1:60 and 1:10, respectively. Human serum (WHO International immunoglobulin reference preparation 67/86) was used as standard control. The diameter of precipitin rings after diffusion time of 50 h (IgG and IgA) and 80 h (IgM) was measured by a calibrated measuring template (Hoechst Pharmaceuticals Ltd., India). The immunoglobulin concentrations were read directly from the table of reference values. Results were expressed in International Units (IU).

Quantitation of B lymphocytes

Lymphocytes were separated from supernatant plasma of heparinized peripheral blood by a Ficoll-hypaque density gradient centrifugation method (Boyum, 1968). For marking of B lymphocytes, the EAC (erythrocyte-antibody-complement) rosette technique as described by Bianco et al. (1970) was followed. Sheep erythrocytes (SRBC) were coated with anti-SRBC antibody (at sub-agglutinating titre) and then with complement. These sensitized SRBC were incubated with the human lymphocytes for 30 min at 37°C. Lymphocytes which bound to ≥ 3 SRBC were counted as rosette. Results are expressed as the percentage of rosetted cells in a total of 400 lymphocytes counted.

Statistical analysis

Results were analysed by Student's t-test.

Results

Table 2 shows the mean values of serum IgG, IgM and IgA levels in 24 KA patients before treatment and thereafter at predetermined intervals during the

Table 2. Serum immunoglobulins (IgG, IgM and IgA) in kala-azar patients and in uninfected controls. Data are mean Ig level (IU/ml) \pm S.E.

| Group (No. studied) | Immuno- globulin class | Period of observation | | | | | | | |
|---------------------------|------------------------------|------------------------------------|------------------------------------|------------------------------------|------------------------------------|------------------------------------|------------------------------------|------------------------------------|------------------------------------|
| | | PT | 1 M | 2 M | 3 M | 4 M | 5 M | 6 M | 8 M |
| KA patients (N = 24) | IgG | 676.29 ^a ± 74.60 | 577.75 ^a ± 63.96 | 524.58 ^a ± 58.61 | 456.83 ^a ± 50.20 | 403.37 ^a ± 44.74 | 335.58 ^a ± 35.33 | 289.25 ^a ± 28.52 | 227.54 ^a ± 17.47 |
| | IgM | 281.45 ^a ± 25.02 | 242.04 ^a ± 21.07 | 206.08 ^a ± 18.58 | 179.58 ^a ± 16.06 | 161.62 ^b ± 15.01 | 158.45 ^b ± 16.51 | 157.66 ^b ± 16.71 | 128.00 ^b ± 11.16 |
| | IgA | 110.04 ^b ± 7.31 | 109.62 ^b ± 5.92 | 113.04 ^b ± 5.85 | 104.87 ^b ± 6.93 | 99.29 ^b ± 5.97 | 96.45 ^b ± 6.33 | 96.87 ^b ± 6.78 | 98.09 ^b ± 5.45 |
| Controls (N = 30) | | IgG = 157.16 ± 7.35 | IgG = 132.56 ± 7.60 | IgG = 103.30 ± 4.15 | | | | | |

PT = pre-treatment; M = month(s) after commencement of treatment; a = t-test significant ($P < 0.01$), b = not significant ($P > 0.05$) in comparison to controls

period of observation for 8 months. Compared with the control value, IgG level was found significantly (t-test, $P < 0.01$) high (about 4.3 fold) in KA patients during active stage of the disease before treatment. A significant (t-test, $P < 0.01$) rise in IgM level (about 2 fold) was also noted before treatment in comparison to controls. During treatment, the IgG level gradually diminished in patients,

Table 3. Circulating B lymphocyte population in kala-azar patients and in uninfected controls. Data represent the mean percentage of B lymphocytes \pm S.E.

| Patient (No. studied) | Period of observation | | | | | | | |
|--------------------------|----------------------------------|----------------------------------|----------------------------------|----------------------------------|----------------------------------|----------------------------------|----------------------------------|----------------------------------|
| | PT | 1 M | 2 M | 3 M | 4 M | 5 M | 6 M | 8 M |
| KA patients (N = 24) | 33.97 ^a ± 0.75 | 32.70 ^a ± 0.59 | 30.72 ^a ± 0.64 | 28.29 ^a ± 0.61 | 26.87 ^b ± 0.45 | 25.22 ^c ± 0.47 | 24.13 ^c ± 0.44 | 23.40 ^c ± 0.58 |
| Controls (N = 30) | 25.66 ± 0.37 | | | | | | | |

PT = pre-treatment; M = month(s) after commencement of treatment; a = t-test significant ($P < 0.01$), b = not significant ($0.02 < P < 0.05$), c = not significant ($P > 0.05$) in comparison to control group

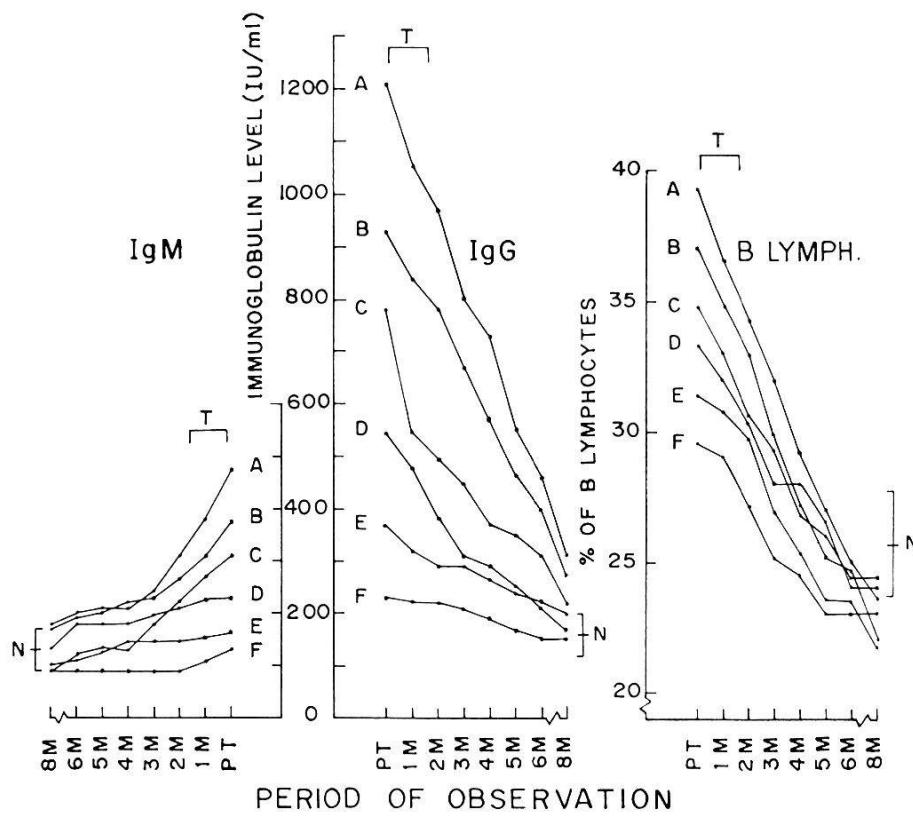


Fig. 1. Immunoglobulin level and B lymphocyte population in 24 kala-azar patients in response to treatment. A-E = mean courses of six equal groups where each group consists of four patients having comparable pretreatment values; PT = pretreatment; M = month(s) after commencement of treatment; N = mean value \pm S.D. of 30 controls; T = treatment, i.e. 2 courses of sodium-antimony-gluconate.

though remained significantly (*t*-test, $P < 0.01$) higher than the normal level after 8 months. It is important to note that level of IgM was restored to normal after 4 months. On the other hand, no such significant difference could be noted in serum IgA levels between KA patients and controls at any point of observation.

Percentage of circulating B lymphocytes in KA patients (estimated sequentially during the period of investigation) in comparison to controls is presented in the Table 3. Before starting of the treatment, a significant (*t*-test, $P < 0.01$) elevation in percentage of B lymphocyte population beyond normal control level was noted. With the clinical improvement following administration of drugs, the B lymphocyte level was restored to normal level after 6 months.

Fig. 1 illustrates the pattern of decrease in serum immunoglobulin (IgG and IgM) levels and B lymphocyte population size in 24 KA patients (represented by 6 equal groups, A–E, each consisting of 4 patients having comparable pretreatment values).

Discussion

The observations made here clearly demonstrate an enormous increase in the level of immunoglobulins, particularly IgG (about 4.3 fold) and to a lesser extent IgM (about 2 fold) in the sera of KA patients, compared to that in controls. No significant change was recorded in IgA level. These observations are in agreement with that of Ghose and Chowdhury (1977), Musumeci et al. (1977), Aikat et al. (1979) and Ghose et al. (1980). Studies made by earlier workers did not trace the levels of IgG and IgM in KA patients throughout their clinical course, specially as they move towards cure in response to treatment, and at different intervals thereafter. Aikat et al. (1979) recorded a significant fall in IgG and IgM levels after therapy when the patients were cured. But present study reveals that along with clinical improvement in response to treatment, IgM fraction returned to normal level after 4 months whereas IgG fraction though showing a gradual fall remained significantly higher (about 1.4 fold) than normal levels even after 8 months. It is known that IgM fraction of immunoglobulin is generally increased though for a comparatively short duration, during the active phase of any infection. Increase in IgG level, however, has been observed by Musumeci et al. (1977) and Volti et al. (1980) not only throughout active phase of the disease process but also for a long period after therapy. Moreover, the immunological changes have been known to vary even among healthy subjects in many tropical areas, and difference of social class may have a marked influence on immunoglobulin levels (Greenwood and Whittle, 1981). Residents of tropical countries, from childhood are continually exposed to microbial organisms and other antibody provoking stimuli from other sources (Higashi and Chowdhury, 1971). Thus the increase of immunoglobulin noted here need not be entirely and specifically attributed to leishmanial infection. It is possible that for hypersynthesis of immunoglobulin in KA, both specific and

non-specific stimulatory mechanisms are operative. But, elevation in the levels of IgG and IgM in KA sera in comparison to that in normal controls of the same endemic area recorded here, may suggest that increase in immunoglobulin fractions during active phase of the disease is perhaps largely due to leishmanial infection. Ghose et al. (1980) considered that marked rise in serum immunoglobulin levels in KA appeared to be due to polyclonal activation of B lymphocytes. Information available till now concerning B lymphocyte population in KA patients is less than adequate and somewhat conflicting. This study showed that B lymphocyte population was markedly elevated during active stage of KA and when the patients were cured it returned to normal. Aikat et al. (1979) also recorded similar results in Indian KA patients. But Carvalho et al. (1981) in patients of American visceral leishmaniasis did not note any change in B lymphocyte population before and after treatment. On the other hand, Rezai et al. (1978) recorded that in majority of KA patients B lymphocyte population was elevated during their active stage and it remained unaltered even after clinical recovery with full course of antileishmanial treatment. Moreover, variation in size of the B cell population in normal healthy subjects is not unknown. Brain et al. (1976) have reported that in Durban, B lymphocyte counts were highest in Indians and lowest in Europeans, with African Negroes showing intermediate values. Similar observation in B lymphocyte population in normal healthy subjects collected from two different areas of India was recorded by Aikat et al. (1979). However, lack of unanimity about information on B lymphocyte population in KA may be due to the likely variation in time of collection of samples during the course of the disease or after cure. The present study seems to reveal a clearer picture, because it recorded the size of B cell population sequentially during the course of the disease and follow-up observations were made even after cure. Besides, a comparison was made with the values of normal controls from the same endemic area.

It is of interest to note that all the patients with high level of immunoglobulin also showed marked increase in the percentage of B cells during the active untreated stage of the disease. It has also been shown in an earlier study (Neogy, 1984) that increase in immunoglobulin levels (more so in IgG than IgM) was associated with corresponding rise in antileishmanial antibody titre. Besides, antibody titre went down progressively commensurate with the gradual fall in immunoglobulin level following treatment and came below the diagnostic level in 5 months in majority of cases. In the present study drug induced clinical improvement revealed a positive correlation between the progressive decline in the B lymphocyte population and immunoglobulin (particularly IgG) level, though the later took a longer time to return to normal.

It should be noted that as the drug SAG was not used on uninfected control, its effects, if any on the immune responses remain unknown. Thus the way polyclonal activation of B lymphocyte, immunoglobulin level and other immunological changes take place following treatment with SAG is not clearly under-

stood. However, the observations made here would seem to suggest that estimation of B cell population may serve as a marker with a prognostic value during the course of the disease.

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