Enzyme Immunoassay of Serum β-2-Microglobulin Levels in Various Histological Forms of Leprosy with Special Reference to Its Elevation in Type I and Type II Lepra Reactions

KUNAL SAHA,1* ACHLA BHATNAGAR,1 V. K. SHARMA,2 AND ASIT K. CHAKRABARTY3

Department of Allergy and Immunology, Vallabhbhai Patel Chest Institute, University of Delhi, Delhi-1100071; Department of Microbiology, Maulana Azad Medical College, New Delhi-110002; and Department of Biochemistry, University College of Medical Sciences, New Delhi-110016, India

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The mean β-2-microglobulin level in serum (3.362 ± 2.494 μg/liter) for 76 leprosy patients, including 9 borderline-tuberculoid, 8 borderline-borderline, 9 borderline-lepromatous, and 16 lepromatous-lepromatous patients and 34 patients with type I or type II lepra reactions, was significantly higher (P < 0.001) than that (2.122 ± 1.844 μg/liter) for 35 normal subjects. It decreased significantly (P < 0.001) as the disease glided down from borderline tuberculoid (3.173 ± 899 μg/liter) to the lepromatous end (1.813 ± 1.391 μg/liter). At the onset of type I or type II reaction, the mean β-2-microglobulin level in serum increased (4.447 ± 2.863 μg/liter), and it remained unchanged (4.433 ± 2.623 μg/liter) after clinical remission. The β-2-microglobulin level in serum decreased in 55.5% of the patients tested after subsidence of reaction. The level was significantly higher in patients with type II reactions (5.433 ± 3.299 μg/liter) than in patients with type I reactions (3.558 ± 2.171 μg/liter).

β-2-Microglobulin (β-2-m), a nonglycosylated polypeptide associated with HLA antigens and also found in serum, has a marked amino acid sequence homology with the CSH domain of immunoglobulin G (IgG) (3). Elevated β-2-m levels in serum in autoimmune diseases, several advanced malignant conditions, and chronic granulomatous and lymphoproliferative disorders suggest the association of β-2-m with polyclonal or monoclonal activation of immune system. Patients in remission rarely show raised β-2-m levels in their sera (14). It is raised in sera of patients with altered glomerular filtration also, as with early rejection of kidney transplants (4, 8, 9, 19, 20, 24). Lepromatous patients show polyclonal B-cell activation (7). Renal involvements, evidenced by low creatinine clearance and histopathological changes, have been reported in both lepromatous and nonlepromatous leprosy patients (18). In this communication we have reported the β-2-m levels in the sera of normal Indian subjects, leprosy patients with various histological forms, and patients at the onset of type I or type II reaction and also after their clinical remission.

Seventy-six leprosy patients (age range, 14 to 70 years; mean age, 42 years), including 34 patients with mild to severe reaction, 7 tribal patients, and 7 female patients (age range, 24 to 56 years), were included in the study. Duration of their illness varied from 3 months to 15 years. Diagnosis was established by examination of clinical features, histopathological study of skin (21), and lepromin testing. There were 9 borderline-tuberculoid (BT), 8 borderline-borderline (BB), 9 borderline-lepromatous (BL), and 16 lepromatous-lepromatous (LL) patients. Some patients received dapsone and clofazimine, others received dapsone and rifampicin, and the remaining patients received the three drugs in combination. Only one patient received dapsone, clofazimine, and prothionamide. Three male histoid patients from the lepromatous group (age range, 35 to 45 years; duration of illness, 3.5 to 15 years) were given dapsone, clofazimine, and rifampicin. Of the 34 patients with reaction (including four females; age range, 15 to 70 years; duration of illness, 1 to 11 years), 25 had BL and LL leprosy and developed type II lepra reactions. The remaining nine patients (BT or BB) developed type I lepra reactions. One 22-year-old male histoid leprosy patient (duration of illness, 2 years) developed a type I reaction. His nodules became red, swollen, and reactivated and showed reversal type reaction. All 34 patients with type I or type II reactions received corticosteroids. Seven patients with type II reactions also received rifampicin, and four patients with type I reactions received dapsone. Thirty-five normal male subjects of similar age group and socioeconomic status constituted the control group. Renal status of the patients and control subjects was not known to us.

One serum sample was collected from each subject. Two samples each were taken from the 34 reactional patients; one sample was taken at the onset of reaction before steroid therapy was begun, and the subsequent sample was taken after the clinical remission of reaction and withdrawal of steroids.

β-2-m levels were determined by a competitive enzyme immunoassay (ABS-75103, Pharmacia Diagnostics, Uppsala, Sweden) in test and control sera. The data were grouped, mean and standard deviations were determined, and a statistical significance test was performed.

The mean β-2-m levels in serum for control and leprosy patients were 2.122 ± 1.844 μg/liter (range, 252 to 6,800 μg/liter) and 3.362 ± 2.494 μg/liter (range, 210 to 11,000 μg/liter), respectively. The difference was statistically highly significant (t = 2.83, P < 0.001) (Fig. 1). Mean β-2-m levels in serum for 9 BT, 8 BB, 9 BL, and 16 LL patients were 3.173 ± 899, 2.708 ± 1,820, 2.130 ± 1,472, and 1.813 ± 1,391 μg/liter, respectively. In the sera of the three histoid leprosy patients, β-2-m levels were 2.080, 1.480, and 1.760 μg/liter (Fig. 2). The differences between the mean levels of β-2-m in serum for controls and BT patients (t = 2.43; P < 0.02) and
between those for BT and LL patients \((t = 4.51; P < 0.001)\) were significant.

The average level of \(\beta-2\)-m in serum for 24 (BL and LL) patients without reaction was 1,955 \(\pm\) 1,449 \(\mu\)g/liter, and that for 34 patients at the onset of reaction was 4,447 \(\pm\) 2,863 \(\mu\)g/liter. This difference was highly significant \((t = 7.46; P < 0.001)\). After the clinical remission of reaction, the level of \(\beta-2\)-m in serum remained almost unchanged \((4,433 \pm 2,623 \mu\text{g/liter})\). Further analysis showed that \(\beta-2\)-m levels in serum decreased in 19 out of 34 (55.5%) patients, increased in 12 (35.6%) patients, and remained unchanged in 3 (8.8%) patients (Fig. 3). The mean concentration of \(\beta-2\)-m in serum was higher in type II reactions than in type I reactions (Table 1).

The mean \(\beta-2\)-m level in serum for Indian subjects (2,122 \(\mu\)g/liter) is comparable to that (2 mg/liter) observed earlier (13). The high (6,800 \(\mu\)g/liter) level observed in the serum of one normal Indian female is not surprising. Similar high values in normal individuals, despite adequate renal functions, have been previously described (20).

The elevation of \(\beta-2\)-m levels in serum has been observed in leprosy patients for the first time. Revillard et al. (20) demonstrated raised \(\beta-2\)-m levels in the sera of patients with sarcoidosis and Crohn’s disease and suggested an association of polyclonal activation of B-lymphocytes with the \(\beta-2\)-m level in serum. They also showed increased \(\beta-2\)-m levels in the sera of patients with autoimmune diseases. Leprosy is a chronic granulomatous disease. Increased immunoglobulin levels in serum and various autoantibodies are found in these patients, symptoms which indicate polyclonal B-cell activation (23).

An alternative explanation of raised \(\beta-2\)-m levels in sera of leprosy patients may be the presence of cold-reacting lymphocytotoxic antibodies in their sera (22), which does not correlate with any of the HLA antigens of the donor cell (S. Naik, B. Kumar, and S. Sehgal, 12th Int. Lepr. Cong., abstr. no. 11, p. 95, 1984). Also, raised levels of the IgM class of antibodies against \(\beta-2\)-m levels in the sera of systemic lupus erythematosus and leprosy patients have been found (1).

A decrease in \(\beta-2\)-m level in serum with the shift of illness from tuberculoid to the lepromatous end (Fig. 2) can be explained by the findings of Revillard, who observed a very high \(\beta-2\)-m level in serum \((4,070 \pm 1,390 \mu\text{g/liter})\) for patients with chronic active hepatitis and a low level for patients with chronic persistent hepatitis \((2,240 \pm 790 \mu\text{g/liter})\). He related the high level of \(\beta-2\)-m in serum observed in patients with chronic active hepatitis to the degree of lymphocyte infiltration of the liver and related the lower level in patients with chronic persistent hepatitis to lesser lymphocyte infiltration.

A heavy lymphocytic infiltration is found in the dural epitheloid cell granuloma of patients with tuberculoid leprosy, which is usually scanty in the histiocytic cell granuloma within the dermis in lepromatous patients (11). Thus, the high \(\beta-2\)-m levels observed in the sera of the BT leprosy patients may be due to the extensive dural lymphocytic infiltration.

During any immune response (e.g., BT leprosy), substantial \(\beta-2\)-m is produced locally in a lymph node, the spleen, or bone marrow, which in turn might be able to affect lymphocytes in the neighborhood and convert these lymphocytes from their nascent forms into suppressor cells (5). Suppressor cell activity is associated with tuberculoid leprosy patients (6). Also, for patients with chronic active hepatitis, steroid therapy usually decreases the degree of inflammation
and lowers the β-2-m level in serum (20). Similar decreases have been observed in β-2-m levels in the sera of 19 of 34 (55.5%) patients with leprosy reactions after treatment with prednisolone (Fig. 3).

The rise of the β-2-m level in serum during the reactional phase of the disease needs explanation. During erythema-nodulosum-leprosum, there is deposition of immune complexes and acute-phase reactants extravascularly at the site of small granulomas (15–17; M. J. Ridley and D. S. Ridley, 12th Int. Lepr. Cong., abstr. no. 11, p. 87, 1984). Child et al. (8) demonstrated a rapid fall of β-2-m and C-reactive protein levels in the sera of some patients of Hodgkin’s disease or non-Hodgkin’s lymphoma after radio- or chemotherapy, which signified remission of the illnesses. Earlier, we demonstrated raised CRP level in serum during and after remission of erythema-nodulosum-leprosum (23).

Estimation of β-2-m levels in serum offered an alternative method for the assessment of glomerular filtration rate. Lepromatous leprosy patients showed proliferative glomerulonephritis, amyloidosis, and renal tubular changes (12). Proteinuria and low clearance of creatinine have been found in nonlepromatous and lepromatous cases (18). Proteinuria, edema, and other biochemical abnormalities have also been observed in the reactional phase of leprosy (25). It is possible then that there may be a loss of functional nephrons

| TABLE 1. β-2-m levels in serum in type I and II lepra reactions |
|-----------------|-----------------|-----------------|
| Lepra reaction  | No. | β-2-m level (µg/liter) in serum (range) |
| type            |     | At onset of reaction | After clinical remission of reaction |
| I               | 9   | 3.558 ± 2.171 (1.680–8.200) | 3.374 ± 1.508 (2.310–7.140) |

* Expressed as mean ± standard deviation. Statistical significance (type II versus type I): at onset, t = 1.92 and 0.05 < P < 0.1; after remission, t = 2.35 and P <0.05. A P value of <0.05 is considered significant.

in these patients (26). Thus, another possible explanation for the high β-2-m levels in the sera of leprosy patients during reaction is the involvement of glomerulin, which warrants a future study of β-2-m levels in the urine of these patients. Bajaj et al. (2) have demonstrated impairment of renal function in leprosy patients during reaction and also during quiescent phase.

LITERATURE CITED