ANTI-NEUTROPHIL CYTOPLASMIC AUTOANTIBODIES IN LEPROSY

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SUMMARY

The objective was to evaluate the frequency of cytoplasmic and peripheral antineutrophil cytoplasmic antibodies (ANCA) in patients with leprosy, and to correlate the presence of ANCA with type and disease activity. Consecutive patients with leprosy were assessed clinically, and IgG ANCA were measured by indirect immunofluorescence. The presence of three of the following was used to assess disease activity: reactive state, fever, new cutaneous lesions, erythrocyte sedimentation rate and C-reactive protein. Sixty-four patients were studied and divided according to the Ridley–Jopling classification: of 38 patients with lepromatous leprosy, eight (21%) had perinuclear (p) ANCA and two (6%) had cytoplasmic ANCA. ANCA titres ranged from 1:20 to 1:320. Of six borderline leprosy patients, one (16%) had p-ANCA. All 20 tuberculoid leprosy patients and 65 healthy control subjects had negative ANCA. There was no correlation between ANCA titres and disease activity in positive patients. ANCA, mainly those with a perinuclear pattern, may be present in leprosy, especially in the lepromatous pole. This disease should be added to the spectrum of diseases with ANCA positivity.

KEY WORDS: Leprosy, Antineutrophil cytoplasmic antibodies.

LEPROSY is a chronic infectious disease caused by *Mycobacterium leprae*, an acid-fast bacillus with high infectivity, low pathogenicity and high virulence. Leprosy is one of the six major infectious disease problems of concern defined by the World Health Organization. Worldwide, 5 500 000 leprosy cases were estimated in 1991 [1]. The American continent contributes 347 066 cases [2]. In Mexico, there were 16 694 registered cases in 1991 [3]. It is estimated that for each case registered, there are one or two cases not reported [1–3]. This disease has a broad spectrum of clinical manifestations, with tuberculoid leprosy (TL) at one end of the spectrum and lepromatous leprosy (LL) at the other. LL is characterized by the virtual absence of T-cell responses to *M. leprae* [4] and it is frequently associated with the presence of autoantibodies such as rheumatoid factor (RF), anticardiolipin antibodies (aCL) and antinuclear antibodies [5] (ANA).

Antineutrophil cytoplasmic antibodies (ANCA) are autoantibodies directed against lysosomal constituents of neutrophils and monocytes, with two major immunofluorescence staining patterns: cytoplasmic (c-ANCA, mainly proteinase 3) and perinuclear [p-ANCA, mainly myeloperoxidase (MPO)] [6]. Until recently, ANCA, especially c-ANCA, were thought only to be associated with granulomatous vasculitic syndromes, mainly Wegener’s granulomatosis [7]. However, the spectrum of ANCA-positive diseases has been broadened as ANCA have been described in rheumatoid arthritis (RA) [8], systemic lupus erythematosus (SLE) [9], ulcerative colitis [10], occupational exposure to silica [11], tuberculosis, subacute bacterial endocarditis [12], invasive amoebiasis [13], reactive arthritis [14], HIV infection [15] and chromomycosis [16].

As leprosy is a chronic granulomatous infection with frequent autoimmune phenomena, we decided to investigate the presence of ANCA specificity in leprosy patients.

MATERIALS AND METHODS

We studied 64 leprosy patients without evidence of other concomitant diseases, mainly SLE, RA and systemic vasculitis. The diagnosis of leprosy was confirmed by bacilloscopy and/or skin histopathology, and patients were classified according to Ridley and Jopling [17]. A reactional state was defined as the newly appearing presence of at least three of the following: erythema nodosum, fever, vasculitic lesions, peripheral neuropathy, high erythrocyte sedimentation rate (ESR, Wintrobe, normal <20 mm/h for females and <15 mm/h for males) and elevated C-reactive protein (CRP). Normal controls consisted of 65 healthy blood donors. All serum samples were collected prospectively and were stored in aliquots at −70°C. CRP was measured by quantitative nephelometry.

ANCA were determined by indirect immunofluorescence with a commercially available kit (INOVA Diagnostics, San Diego, CA, USA) that makes use of ethanol-fixed human neutrophil substrate slides. Sera were applied using doubling dilutions of fluorescein isothiocyanate-labelled goat anti-human IgG, starting at a 1:10 dilution. As a control, known positive and negative sera with c-ANCA or p-ANCA patterns were included in each assay. All patients were screened for hepatitis B (HBsAg) and C (ELISA), and HIV infection (ELISA), with negative results in all cases.

RESULTS

Of the 64 patients studied, 49 were men and 15 women with a mean age of 38.4 yr (24–58) and a mean disease duration of 8.3 yr. The diagnosis was confirmed by lympathic fluid bacilloscopy in 44 patients and/or skin histopathology in 39 cases. All patients were on
multidrug therapy (dapsone, clofazimine and rifampin; MDT). Thirty-eight patients had LL, six had borderline leprosy (BL) and 20 had the tuberculoid form. Nineteen patients were in a reactional state at the time of the study, 16 of them lepromatous and three borderline, characterized in 10 by erythema nodosum, six with arthritis and neuropathy, two with Lucio’s phenomenon (generalized necrotizing vasculitis with fever in a lepromatous patient) and one was in a reversal reaction (inflammatory reaction seen at the onset of therapy, usually seen as peripheral neuropathy).

Table I shows the data obtained from the patients studied. As seen, ESR was elevated in 20/38 LL patients (53%), 3/6 BL patients (50%), 3/20 TL patients (15%) and 2/65 controls (3%); CRP was high in 18/38 (47%) LL patients, 4/6 BL patients (66%), 2/20 TL (10%) and 3/64 controls (4%).

With regard to ANCA, 8/38 LL patients (21%) had p-ANCA and 2/38 (5%) had c-ANCA. One of six BL patients (16%) had p-ANCA. Titres ranged from 1:20 to 1:320, without any correlation between the presence and/or titre of ANCA and a reactional state, or with ESR and CRP levels, since 5/19 with a reactional status (26%) and 5/25 inactive patients (20%) of the lepromatous pole were ANCA+. The higher titres (1:320) were found in both patients with Lucio’s phenomenon. No ANCA were found in any of 20 TL patients nor in the 65 controls.

**DISCUSSION**

These studies were undertaken to examine the presence of another type of autoantibody in a disease with well-established associated autoimmunity: leprosy. ANCA were determined in 64 patients with various forms of leprosy and in healthy individuals. Over one-fifth of patients with LL and a smaller percentage of patients with BL had a positive p-ANCA pattern, whereas a positive c-ANCA pattern was identified in 5% of LL patients.

Even though leprosy is caused by a single infectious agent, *M. leprae*, its clinical manifestations occupy a broad spectrum. At one pole, patients with TL have mild skin and nerve lesions, consistent in hypochromic plaques with hypo- or anaesthesia and hypohidrosis, where bacilli can rarely be identified, and show strong cell-mediated immune responses to *M. leprae*. At the opposite pole, patients with LL have diffuse and severe skin and nerve infiltration with extensive bacterial load (up to 10 billion microorganisms per gram of host tissue) [18], and anergy to *M. leprae*, whereas immune reactivity to other antigens is normal [19].

Patients with LL have a high incidence of other autoantibodies, such as RF (13–60%) [20] and ANA (0–30%) [21, 22]. It has been suggested that antibodies directed against mycobacterial phospholipids could cross-react with the phosphate backbone of DNA [23], but this has been denied by others [21]. Anticytoskeletal antibodies have also been described in LL with cross-reactivity between mycobacterial proteins and human intermediate filament protein subunits [24].

Heat shock proteins (HSP) are phylogenetically conserved with high homology between human and microbial HSP, mainly with mycobacterial HSP60 and HSP65 [25]. Therefore, HSP have been focused on as targets of autoimmune responses due to possible cross-reactivities and because HSP are immunodominant antigens. Although there are no clinical reports of immunological cross-reaction between HSP and MPO, homology in the amino acid sequence has been reported [26]. Thus, reactivity to HSP could be implied in the genesis of ANCA in leprosy and other chronic infectious diseases.

Antibodies to the 29 kDa serine protease 3 or PR3 are considered to have high sensitivity for Wegener’s granulomatosis (c-ANCA) [27]; in addition, they have been employed to distinguish between active and inactive disease. In contrast to initial reports that showed c-ANCA reactivity directed against protease 3, it has recently been reported that classic c-ANCA-positive sera may contain antibodies directed against other antigens [28]. However, c-ANCA are also found in necrotizing glomerulonephritis, different forms of systemic vasculitis and SLE. On the other hand, p-ANCA are present in idiopathic crescentic glomerulonephritis, Churg–Strauss angiitis and microscopic polyarteritis nodosa. Several studies have stressed the clinical significance of ANCA positivity in necrotizing vasculitis and rheumatic pathologies [6–9]. However, ANCA are also present in miscellaneous conditions, including ulcerative colitis [10], occupational exposure to silica [11], HIV infection [15], subacute bacterial endocarditis [12], reactive arthritis [14], tuberculosis and chromomycosis [16].

Although the origin of autoantibodies in leprosy...
and other chronic inflammatory disease is obscure, it could be the reflection of polyclonal B-cell activation by bacterial components. However, polyclonal B-cell activation rarely results in the production of IgG antibodies. Thus, it is more likely that ANCA in leprosy patients are due to B-cell stimulation by antigen with the participation of T cells. T-cell anergy in LL involves Th1 cells with the resulting lack of interferon-γ-mediated macrophage activation, necessary to eliminate intracellular M. lepra. Even though there is no direct evidence for a role of Th2 cells in the pathogenesis of LL, Th2-like cytokines have been found in the disease.

Th2 activation could be induced after the release of neutrophil components in the disease-associated lesions and could be facilitated by the long duration of the disease. The presence of a reactive state could be another factor enhancing the production of autoantibodies as the levels were present in both patients with Lucio’s phenomenon. However, we did not find any other relationship of ANCA positivity with disease activity. As it has been recently shown that certain antibiotics (minocycline) can induce p-ANCA [29], it could be possible that MDT could also induce these autoantibodies. In conclusion, the production of these autoantibodies could be related to a prolonged and continuous immunological challenge by a foreign stimulus (microorganisms or chemicals).

In summary, we report that ANCA, mainly those specific against MPO, are present in the serum of patients on the lepromatous pole of leprosy. Their role in this entity remains to be elucidated. Leprosy must be added to the disease spectrum that may present ANCA positivity.

REFERENCES

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