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Expression of a common idiotype PR4 in the sera of patients with leprosy

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SUMMARY

The sera of 187 patients from across the leprosy spectrum were screened for the expression of the PR4 idiotype, which was first identified on a human hybridoma-derived monoclonal antibody from a patient with leprosy and found to react with the *Mycobacterium leprae* phenolic glycolipid and a variety of polynucleotides. Sixty per cent (51 out of 85) of patients with lepromatous leprosy (LL), 66% (33 out of 49) with borderline lepromatous (BL) disease, 47% (14 out of 30) with borderline tuberculoid (BT) leprosy, and 56% (13 out of 23) of tuberculoid (TT) patients were found to have significantly elevated titres of the PR4 idiotype in their sera compared with endemic controls, irrespective of the presence or absence of endemic malaria. Sera from 52 patients with tuberculosis were also screened as a control for mycobacterial infection. The PR4 idiotype was significantly elevated in 37% (19 out of 52) of these patients. No correlation between idiotype and serum immunoglobulins IgG and IgM was found, indicating that the concentrations of idiotype levels in sera were not merely a reflection of changes in serum immunoglobulin levels. It is hypothesized that the expression of the PR4 idiotype is due to certain germline genes preferentially expressed rather than being the result of polyclonal B cell activation.

Keywords leprosy autoantibodies tuberculosis idiotypes

INTRODUCTION

Many immunological abnormalities have been described in leprosy, including the presence of a wide variety of autoantibodies against many organ-specific and non-organ-specific antigens. Although this phenomenon is most prevalent in the lepromatous form of the disease, it also extends across the spectrum to the tuberculoid form (Masala *et al.*, 1979; Bullock *et al.*, 1982). Whether these autoantibodies represent the expression of a limited number of idiotypes on cross-reactive antibodies stimulated by *Mycobacterium leprae* infection or a polyclonal stimulation of many different autoantibodies, is not known (Zumla, 1990). The finding that a leprosy-derived human monoclonal antibody (MoAb) PR4 bound to several autoantigens (Zumla *et al.*, 1988; Locniskar *et al.*, 1988) prompted us to probe the origins of these autoantibodies. The PR4 idiotype was first identified on a human hybridoma-derived MoAb from a patient with leprosy, and was shown to bind the *M. leprae*-specific phenolic glycolipid-1 and a variety of polynucleotides (Zumla *et al.*, 1988). Idiotype PR4 has also been identified in the sera of patients with several autoimmune rheumatic diseases (Williams *et al.*, 1988). We report the detection of the PR4 idiotype in the serum of patients with leprosy and pulmonary tuberculosis.

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SUBJECTS AND METHODS

Human–human hybridoma production

Human MoAbs were produced from human–human hybridomas derived from polyethylene-glycol-mediated fusion between lymphocytes from a Guyanese patient with lepromatous leprosy (LL) and the lymphoblastoid cell line GM4672 (Locniskar *et al.*, 1988). Cloned IgM kappa secreting hybridoma PR4 reactive to phenolic-glycolipid-1, poly(ADP-ribose), ssDNA and tissue antigens (Zumla *et al.*, 1988), was selected for study.

Production of rabbit anti-idiotype PR4

Rabbit anti-idiotype PR4 was prepared as described (Locniskar *et al.*, 1988). The polyclonal rabbit antiserum was rendered idiotype specific by extensive absorption over a pooled human IgG and IgM affinity column until no anti-human immunoglobulin activity was detectable. The polyclonal anti-idiotype was then absorbed onto, and subsequently eluted from, a PR4 affinity column. The purified anti-idiotype PR4 was inhibited

Table 1. Idiotype PR4 concentrations ($\times 10^3$ ng PR eq/ml) in the sera of patients with leprosy and tuberculosis

Diagnosis and origin	*n*	Range	Mean	s.d.	Median	*n* > mean + 2 s.d.	*n* > mean + 3 s.d.
Papua New Guinea							
LL							
Highlands	48	0–330	83	75	62		
Coastal	37	0–300	91	85	75		
Total	85	0–330	87	80	70	51 (60%)	41 (48%)
BL							
Highlands	19	0–270	101	80	90		
Coastal	30	0–250	106	88	110		
Total	49	0–270	104	84	90	33 (66%)	28 (56%)
BT							
Highlands	8	0–120	27	40	10		
Coastal	22	0–130	64	47	70		
Total	30	0–130	54	48	55	14 (47%)	12 (40%)
TT							
Highlands	4	5–100	55	38	38		
Coastal	19	2–180	62	53	53		
Total	23	2–180	60	50	50	13 (56%)	8 (33%)
Controls	30	0–50	17	12	15	3 (10%)	1 (3%)
United Kingdom							
Pulmonary tuberculosis	52	0–400	32	85	55	19 (37%)	10 (20%)
Controls	30	0–60	7	11	5	1 (3%)	1 (3%)

LL, lepromatous leprosy; BL, borderline lepromatous; BT, borderline tuberculoid; TT, tuberculoid leprosy.

from binding to its homologous PR4 idiotype by phenolic glycolipid-1, poly(ADP-ribose) and a variety of polynucleotides indicating that the anti-idiotypes reacted with structures at or near the antigen binding sites.

Selection of serum samples

Leprosy patients. Serum samples from 187 Papua New Guinean patients with leprosy, living in malarious or non-malarious regions of Papua New Guinea, were used. Table 1 shows the number of serum samples from across the leprosy spectrum. These were selected at random from a larger selection of sera as part of a study described elsewhere (McAdam *et al.*, 1977). The patients were examined independently by two clinicians and by skin biopsy before their diagnostic category was assigned. Togoba is a leprosy hospital located near Mount Hagen in the non-malarious eastern highland region of Papua New Guinea, and Aitape Hospital is on the north coast, where malaria is endemic. Selection of control sera was carefully considered since anti-ssDNA antibodies occurring in malaria infections have been well documented (Adu *et al.*, 1982; McAdam *et al.*, 1983). To serve as endemic controls, 30 serum samples from patients known to have low titres of anti-malarial antibodies were selected from a non-malarious highland area of Papua New Guinea (near Aseki). Serial serum samples from two LL patients undergoing erythema nodosum leprosum reactions were also screened for idiotype PR4 levels.

Tuberculosis patients. Fifty-two patients of multi-ethnic origin with pulmonary tuberculosis were also studied. This group included Caucasian ($n=22$), and Asian or African ($n=30$) patients, none of whom had received more than 2 months of specific treatment. Thirty-two serum samples from normal multi-ethnic (including Asian and African) individuals in London, England, served as non-endemic controls.

Measurement of serum immunoglobulins

All test serum samples were screened for IgG and IgM concentrations using immunoturbidity on the Cobas Fara (Roche) centrifugal analyser. Serum samples were diluted 1/40 in phosphate-buffered saline (PBS) containing 10% polyethylene glycol. Rabbit anti-IgG or IgM (Roche) were added and a reference calibration curve calculated from light absorption at 350 nm. The respective immunoglobulin concentrations in mg/ml were measured against this curve.

Screening for the PR4 idiotype

This was performed using an indirect ELISA as previously described (Williams *et al.*, 1988). In brief, 96-well Immulon 1 (Dynatech Laboratories, Alexandria, VA) polystyrene plates were coated on one-half with affinity purified rabbit anti-PR4 in carbonate–bicarbonate coating buffer (CB) (pH 9·6) overnight at 4°C. The other half of the plate was coated with rabbit anti-Freund's incomplete adjuvant (anti-FIA) in CB only, at an immunoglobulin concentration equivalent to that of the rabbit anti-PR4. The rabbit anti-FIA had previously been absorbed on a pooled human immunoglobulin column in parallel with the rabbit anti-PR4. After blocking the plates at room temperature

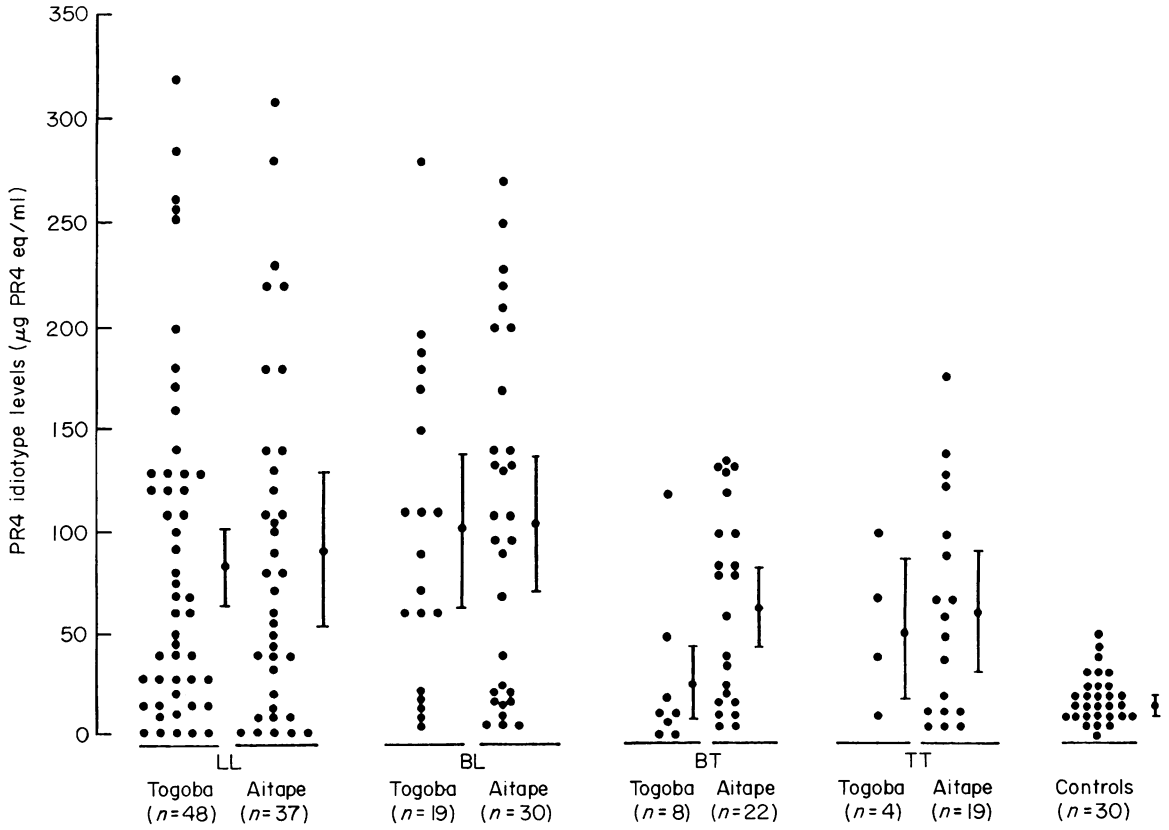

Fig. 1. Idiotype PR4 levels in the sera of patients with lepromatous leprosy (LL); borderline lepromatous (BL) leprosy; borderline tuberculoid (BT) leprosy; tuberculoid (TT) leprosy from the Togoba and Aitape hospitals in Papua New Guinea; and Papua New Guinean controls. Means and 2 s.e.m.

for 1 h with 2% bovine serum albumin (BSA) in PBS, test serum samples diluted in 2% BSA–PBS–Tween were added in duplicate to both anti-idiotype-coated and anti-FIA-coated wells. Every plate had the standard MoAb PR4 curve, consisting of doubling dilutions of the affinity-purified MoAb PR4 added to normal sera (pre-tested as not having antibodies to ssDNA and PGL-1). The final MoAb PR4 dilutions ranged from 500 ng/ml to 16·5 ng/ml diluted in normal sera (1/800 final). A panel of seven normal sera served as reference negative controls. The test sera and normal sera were used at a final dilution of 1/800. After 2 h of incubation, the plates were washed three times with PBS–Tween and 100 μl of alkaline-phosphatase-labelled goat anti-human IgG (Fab'_2 fragments) and IgM in PBS-Tween were added to each well. The plates were read at an absorbance of 405 nm on a Dynatech ELISA MR580 reader with the machine blanked on wells coated with anti-FIA and incubated with sample dilution buffer alone. The arithmetic means of the optical density values of the test samples were subtracted from the means of the background values. The idiotype content of each test sample was read off the respective standard PR4 curve. The idiotype serum concentrations were expressed as equivalents/ml of MoAb PR4.

Statistical analysis

Group means were tested by Student's two-tailed *t*-test. Regression analyses were done by the method of least squares.

RESULTS

Idiotype PR4 frequency in test sera

The descriptive statistics, which include the range, mean, median, and s.d. for the concentration of idiotype PR4 in the sera of leprosy patients, tuberculosis patients and controls is shown in Table 1. For the Papua New Guinean control subjects, the mean idiotype value was 17 μg PR4 eq/ml, and only 5 μg for the UK controls. From Table 1 it is apparent that leprosy patients from all classes have significantly greater idiotype PR4 levels than the endemic controls ($P > 0{\cdot}001$). No significant difference was seen between the multi-bacillary groups with LL and borderline lepromatous (BL) leprosy. However, patients at the lepromatous end of the spectrum tend to have higher levels than the paucibacillary end of the spectrum ($P = < 0{\cdot}05$), although the number of patients from the tuberculoid end of the spectrum was small. A high frequency of patients with tuberculosis (37·5%) also had significantly elevated concentrations of PR4 idiotype. It also appears from Table 1 that patients throughout the leprosy spectrum from malarious zones (Aitape) had higher idiotype levels than those from non-malarious areas (Togoba). However, statistically there was no significant difference between the two groups, using Student's two-tailed *t*-test. Figure 1 shows the distribution of idiotype PR4 levels in the leprosy sera screened. The mean and 2 s.e.m. for each group are represented in the scattergram. The upper limit of normal was set at 42 μg PR4 eq/ml for the Papua New Guinean controls.

This represented 2 s.d. above the mean. Only three out of 33 (10%) of the normals from Papua New Guinea had levels above the upper limit. Sixty per cent (51 out of 85) LL patients, 66% (33 out of 49) BL patients, 47% (14 out of 30) borderline tuberculoid (BT) patients and 56% (13 out of 23) tuberculoid leprosy (TT) patients had significantly elevated idiotype PR4 levels (Table 1). The upper limit of normal for the UK controls was set at 30 μg PR4 eq/ml above the mean+2 s.d. of this population. One of the 32 controls (3%) had an elevated PR4 level, compared to 37% of the patients with tuberculosis (Table 1). The serum of the patients from whose lymphocytes the MoAb PR4 was derived, had significantly elevated PR4 values (220 μg PR4 eq/ml).

Correlation with total IgG and IgM

There was no significant correlation between the PR4 idiotype levels and serum IgG, IgM or total IgG/IgM (data not shown; $r=0{\cdot}13$, $0{\cdot}21$, $0{\cdot}17$, respectively, and significance values all $P<0{\cdot}1$). Thus it is apparent that the levels of idiotype PR4 detected in the sera represent quantitative changes in idiotype levels and are not due to differences in serum immunoglobulin concentrations within different samples.

DISCUSSION

This is the first report describing the finding of a common idiotype in the sera of leprosy patients from across the leprosy spectrum. The observations of this study extend those of Mackworth-Young, Sabbaga & Schwarz (1987) who analysed idiotypes on human MoAbs derived from patients with systemic lupus erythematosus and leprosy, and suggested that the expression of these idiotypes was more closely related to a polyclonal B cell activation. Common idiotypes have been detected in inbred lupus mouse strains such as the idiotype H130 found in MRL-lpr/lpr mice (Rauch *et al.*, 1982), and in the outbred human population on anti-tetanus antibodies, anti-bovine casein antibodies, and a variety of autoantibodies (reviewed by Isenberg & Shoenfeld, 1986).

The expression of the PR4 idiotype is probably not simply the result of polyclonal B cell activation. The PR4 idiotype was found in 59% of the sera of patients from across the leprosy spectrum, and was more frequently found in LL/BL patients irrespective of whether patients lived in areas of malarial endemicity on the coast or above the altitude for malarial transmission in the highlands. Malaria is another disease in which polyclonal B cell activation occurs and the presence of autoantibodies has been well documented (Adu *et al.*, 1982; McAdam *et al.*, 1983). The PR4 idiotype was found at a low prevalence in the highland Papua New Guinea control subjects, a group with the same incidence of malaria as the highland leprosy population. However, it was only found in 15% of patients with Sjögren's syndrome (Williams *et al.*, 1988), a disorder commonly associated with hypergammaglobulinaemia. This too argues against idiotype PR4 expression simply reflecting polyclonal B cell activation. The PR4 idiotype was detected at a concentration more than 2 s.d. above the London controls in 37% of the sera derived from patients with tuberculosis. This compares with the figure of 60% for another common DNA antibody idiotype, 16/6, in the same disease group (Sela *et al.*, 1987). This idiotype was first found on a human hybridoma-derived monoclonal from a patient with systemic lupus erythematosus. The PR4 idiotype has also been found in up to 71% of patients with systemic lupus erythematosus and 40% of those with rheumatoid arthritis (Williams *et al.*, 1988). Idiotype PR4 expression did not correlate with disease activity in systemic lupus erythematosus and was not found more frequently in the healthy first-degree relatives compared with normal controls.

The evidence may imply that certain germline genes are preferentially expressed in the human population. The stimulus that leads to their expression and the evolutionary pressure leading to their conservation remain speculative. These autoreactive antibodies may represent a good starting point for somatic mutation towards higher affinity idiotypes. Their persistence in leprosy may reflect a block in this maturational pathway or a prolonged presence of mycobacterial antigens, thereby foiling the selection process of somatic mutation which depends on decreasing availability of antigen. Alternatively, persistence of PR4 may represent in part the polyclonal drive of persistent mycobacterial components. It seems unlikely that screening for elevated levels of the PR4 idiotype in the sera of leprosy patients will have diagnostic value, since this idiotype has been found in patients with various autoimmune disorders (William *et al.*, 1988) and tuberculosis. Further studies are needed to determine whether common idiotypes such as the PR4 idiotype play an important role in immunoregulation and pathogenesis of leprosy.

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