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Specific IgE response in patients with brucellosis

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SUMMARY

In the search to find discriminative serological markers to differentiate between patients with acute brucellosis and those with chronic brucellosis, an enzyme-linked immunosorbent assay (ELISA) was used to determine and compare the brucella-specific IgE response in 80 sera from patients with acute brucellosis, 37 sera from patients with chronic brucellosis, 26 sera from patients with positive blood cultures for bacteria other than brucella and 51 sera from healthy controls. The IgE findings were compared to brucella-specific IgG, IgM, IgA and IgG_{1-4} demonstrated by ELISA, and to microagglutination test (MAT) results. Elevated (positive) antibrucella IgE titres were detected in 89 and 81% of sera from patients with acute and chronic brucellosis respectively. The predominant antibodies found in patients with acute brucellosis were of the IgG, IgM, IgA, IgE, IgG_1 and IgG_3 types while in chronic brucellosis IgG, IgA, IgE and IgG_4 were found. Although IgE can be detected in patients with brucellosis, it does not discriminate between the acute and chronic stages of the disease.

INTRODUCTION

Studies of the immunoglobulin (Ig) isotype response in brucellosis have been directed towards finding diagnostic and/or prognostic markers for this disease. Most reports have concerned antibrucella IgG, IgM and IgA antibodies [1–5] in relation to their diagnostic value as well as in differentiating between the acute and chronic stages of the disease [6–10]. In addition, changes in the levels of these isotypes was used as a means to predict relapses in a follow-up study [11]. While in a recent study of brucella-specific subclasses of IgG_{1-4} some differences in the profiles of Ig between acute and chronic brucellosis were noted [12], a definite discriminative Ig marker remains to be determined.

Most of the studies on IgE antibodies in infectious diseases have been in relation to parasitic infections [13, 14] with few papers on bacterial [15–17], viral [18–20], or fungal [21] disorders.

Studies on the brucella-specific IgE response in patients with brucellosis are few [22]. The present study was undertaken to determine the brucella-specific IgE

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response in sera of patients with acute and chronic brucellosis by ELISA in an attempt to find an antibody marker that differentiaties between the two stages of the disease.

MATERIALS AND METHODS

Sera

Serum specimens were obtained from 80 patients (sporadic cases) with acute brucellosis (signs and symptoms < 2 months), who had positive blood cultures for *Brucella melitensis*, 37 patients with chronic brucellosis (signs and symptoms > 1 year), 26 patients with positive blood cultures for bacteria other than brucella (4 *Escherichia coli*, 4 *Klebsiella pneumoniae*, 4 *Salmonella* spp., 4 *Pseudomonas* spp., 4 *Enterobacter* spp., 2 *Moraxella* spp. and 4 *Haemophilus* spp.) and 51 sera from apparently healthy controls. All patients and controls were adults with an age range of 20–50 years (mean, 30 for patients and 32 for controls). The criteria for the diagnosis of brucellosis and the clinical and laboratory findings in these patients have been described elsewhere [23]. All sera were stored at −70 °C until tested.

Estimation of brucella-specific IgE by ELISA

Optimum conditions for the ELISA were predetermined for the IgE assay. The 96-well immunoplate (Nunc, Denmark) was coated overnight at room temperature with 50 μl of carbonate buffer (pH 9·6) containing the optimum concentration of pre-titrated antigen from heat-killed *B. melitensis* as previously reported [9]. Fifty μl, in duplicates, of patients and control sera, diluted 1:50 in phosphate buffered saline pH 7·4 containing 0·5% Tween 20 and 0·5 M-NaCl (PBST-NaCl), were added to proper wells and the plates were incubated at 4 °C overnight. After washing with PBST-NaCl, 50 μl of the predetermined concentration of rabbit-anti-human IgE (Behring, West Germany) was added and the plates incubated at 37 °C for 2 h. The plates were washed, and 50 μl of peroxidase-conjugated swine-anti-rabbit immunoglobulins (Dako Patts, Denmark) were added to wells and the plates incubated at 37 °C for 2 h. After washing 50 μl of ortho-phenylene diamine-HCl substrate (Sigma Chemical Co. USA) were added to each well. The plates were incubated in the dark at room temperature for 15 min, and read at optical density (OD) 492 nm using the Titertek Multiscan spectrophotometer (Flow Laboratories, Scotland). Known positive and negative sera were included in each plate and 10% or less variation in optical density was allowed for these controls amongst runs; otherwise all specimens on the plates were tested again. A positive (elevated) IgE result was recorded when the test specimens revealed an ELISA OD 492 $\geqslant$ 2 S.D. above the mean OD of the control specimens.

Estimation of brucella-specific IgG, IgM and IgA by ELISA

The methods were as previously reported [5]. Briefly, 50 μl of serum specimens diluted twofold from 1:100 to 1:12800 in PBST-NaCl were added to appropriate wells of immunoplates precoated with brucella antigen. The plates were incubated at 37 °C for 2 h, washed and alkaline phosphatase conjugated anti-human IgG or IgM or IgA (Sigma) was added to designated wells and incubated at 37 °C for 2 h. After washing, P-nitrophenyl phosphate (Sigma) was added and allowed to

react at 37 °C for 45 min and the plates were read at 405 nm. The antibody titres in the tested serum were calculated using a cut-off optical density equal to or around (within 10%) that obtained at a 1:100 dilution of a known negative serum specimen included in each run of the assay.

Estimation of brucella-specific IgG_{1-4} subclasses

The antibrucella IgG_{1-4} subclasses were determined as previously reported (12). Briefly, 50 μl of serum specimens (diluted 1:100 in PBST-NaCl) were added in duplicates to appropriate wells, of immunoplates precoated with brucella antigen, and incubated at 37 °C for 1·5 h. After washing, 50 μl of an optimum dilution of monoclonal mouse anti-human IgG_1 or IgG_2 or IgG_3 or IgG_4 (In Vitro Export reagents, Fort Lauderdale, FL, USA) were added to appropriate wells and incubated at 37 °C for 1·5 h. After washing, rabbit anti-mouse IgG conjugated to alkaline phosphatase (Sigma) was added to each well and the plates incubated at 37 °C for 1·5 h. After further washing, substrate was added and allowed to react at 37 °C for 45 min and the plates then read at OD 405 nm. A positive (elevated) result was considered when the test specimen revealed an ELISA OD 405 $\geqslant$ 2 S.D. above the mean OD of the 1:100 dilution for the respective IgG subclasses in serum specimens of the controls.

Brucella microagglutination test (MAT)

The MAT was performed as described earlier [9] using *B. melitensis* antigen of strain 16M (courtesy of National Veterinary Services laboratories, Ames, Iowa, USA).

Culture of clinical specimens

The culture and identification of brucella and other microorganisms from clinical specimens was performed according to established procedures.

Statistical analysis

Measured antibody titres, for IgG, IgM and IgA, were transformed to the base 10 logarithm for analysis and the geometric means were compared by using *t* test. The arithmetic means were used in the results of OD for IgE and IgG subclasses and comparisons between the different study groups were also analysed by the *t* test.

RESULTS

The serum IgE results according to the different groups of the study population are presented in Fig. 1. The arithmetic means $\pm$ S.D. of IgE OD were 273 ± 122 for patients with acute brucellosis, 240 ± 97 for patients with chronic brucellosis and 96 ± 27 for all the controls. The IgE OD geometric means for patients with brucellosis were significantly different ($P < 0{\cdot}01$) from those in controls but no significant difference ($P > 0{\cdot}05$) was found between the two control populations.

The results of elevated anti-brucella antibodies in various study groups are presented in Table 1. Patients with acture brucellosis showed elevation in anti-brucella IgG, IgM, IgA, IgE, IgG_1 and IgG_3 antibodies while patients with chronic brucellosis showed elevation in IgG, IgA, IgE and IgG_4.

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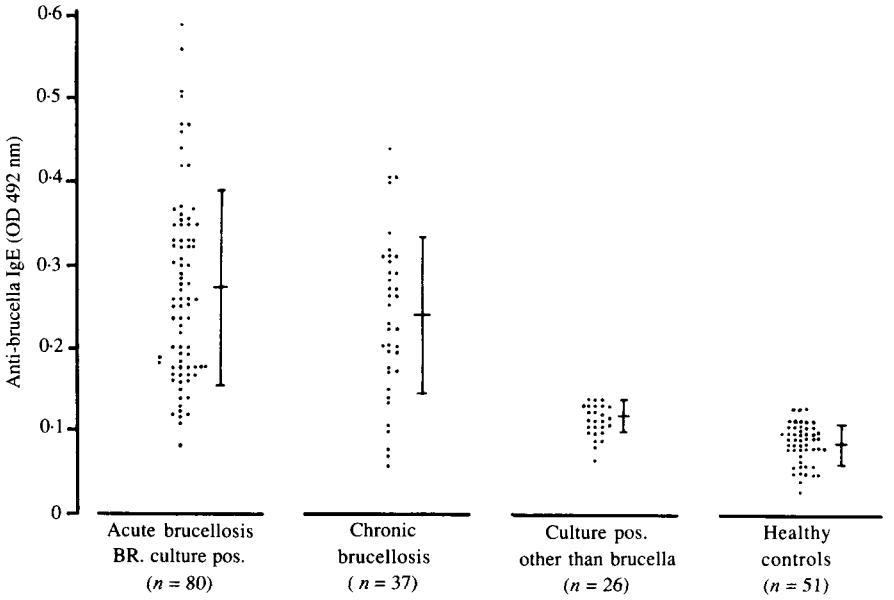

Fig. 1. Serum IgE results in different groups of the study population.

Table 1. *Elevated anti-brucella antibodies in patients with brucellosis*

Anti-brucella antibodies	Titre	Percent of individuals with elevated antibodies in Acute (n = 80)	Chronic (n = 37)	Controls (n = 77)
MAT*	⩾ 80	98	51	0
IgG	⩾ 800	96	100	4
IgM	⩾ 400	98	29	0
IgA	⩾ 200	98	100	0
IgE	NA	89	81	0
IgG_1†	NA	96	43	0
IgG_2†	NA	16	29	0
IgG_3†	NA	92	29	0
IgG_4†	NA	20	81	0

* Microagglutination test.

† IgG_{1-4} subclasses were tested in 25 patients with acute brucellosis, 21 patients with chronic brucellosis and 34 controls.

NA, Not applicable

The results from 25 patients with acute brucellosis and 21 patients with chronic brucellosis whose specimens were simultaneously tested for all the Ig isotypes are presented in Fig. 2. The findings in relation to the elevated antibody isotypes were almost similar to those noted in Table 1 for patients in the acute and chronic brucellosis.

None of the controls showed elevation in antibody isotypes except in IgG where only 4% of the controls had titres equal to 1600. The MAT gave more positive results in patients with acute (98%) than those with chronic (51%) brucellosis.

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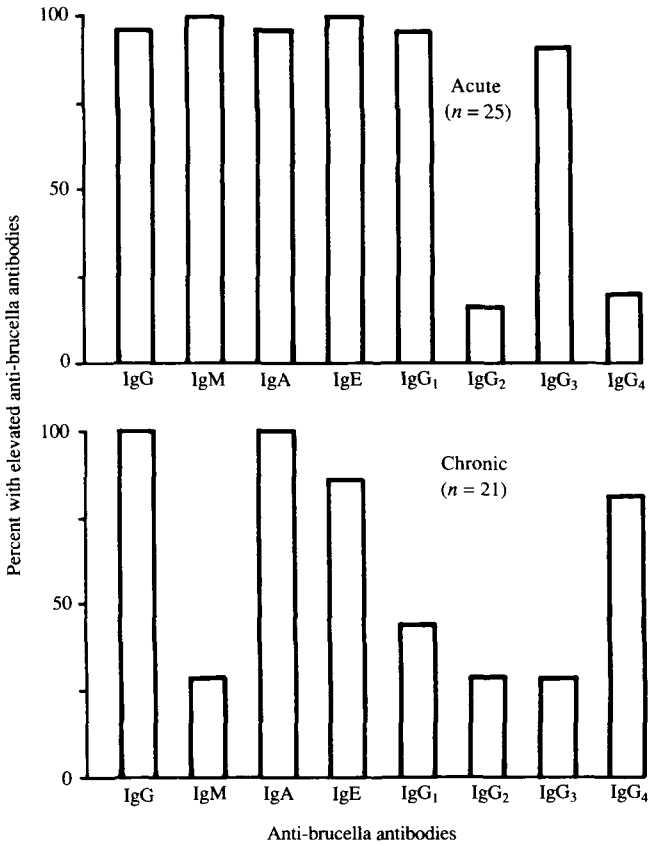

Fig. 2. Immunoglobulin isotypes in 25 patients with acute brucellosis and 21 patients with chronic brucellosis.

DISCUSSION

This work shows that antibrucella IgE antibodies are detectable by ELISA in the majority of patients with brucellosis indicating that this isotype is part of the human antibrucella humoral response. This corroborates earlier findings [22].

The demonstration of the different specific classes or subclasses of antibody response in brucellosis has been suggested as useful both in the diagnosis and prognosis of the disease and in elucidating differences between the acute and chronic stages of brucellosis [6–10]. Patients with acute brucellosis show elevation in brucella specific IgM alone (in around 3% of the patients) or together with IgG and IgA antibodies. While in patients with chronic brucellosis, only IgG and IgA antibodies are consistently elevated [3, 9]. Subclasses of IgG_{1-4} antibrucella antibodies were also studied in patients with acute and chronic brucellosis, revealing predominance of IgG_1 and IgG_3 antibodies in the acute group and IgG_4 in the chronic group [12]. In the present study, however, no distinction could be found between patients with acute and chronic brucellosis based on IgE response, as both groups showed almost similar high incidence in the proportion of patients with elevated IgE. The post treatment monitoring of antibrucella IgG, IgM, and IgA was reported to be helpful in predicting those patients at risk of relapses [11].

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Whether IgE would be useful in this regard remains to be investigated in a follow up study.

Few studies have been reported on the relationship of anti-bacterial IgE antibodies and the associated disease. Associations or correlations were found between severe *Staphylococcus aureus* infection and the presence of IgE anti-staphylococcal antibodies [17], and between atopy and the level of anti-tetanus and anti-diphtheria IgE antibodies [16]. High titres of IgE anti-SK antibodies are said to indicate a poor prognoses in children with nephropathia [15]. In brucellosis, the functional significance of brucella-specific IgE response is unknown. Escande and Serre [22] noted that the specific IgE appears after IgM and before IgG antibodies in patients with brucellosis. They also found no correlation between the brucella-specific IgE antibodies and the level of IgG or IgM, the clinical manifestations, the severity of the disease or the level of total serum IgE. One patient with a high level of total serum IgE had no IgE anti brucella antibodies. In the present study, no association of disease severity to IgE response was observed [22]. In addition, there was no association between the IgE response and any of the classes or subclasses of the Ig tested.

The specificity of the brucella-specific IgE assay was very high (100%). None of the control specimens showed a positive result when the upper limit cut-off was set at 2 S.D. above the mean OD for all control specimens. The sensitivity of the assay was also relatively high as elevated levels of antibody were found in 89 and 81% of patients with acute and chronic brucellosis respectively. In those patients with lower specific IgE antibody values the total IgE levels were also within the normal range. None of the patients was using immunosuppressive drugs or steroids and the other Ig responses to brucella antigens were high. It is possible that the presence of competing antibodies of the IgG, IgM and IgA isotypes may block antigenic sites for IgE, since they are present at larger quantities and may thus limit the sensitivity of the IgE detection technique as has been reported for viruses [20].

In conclusion antibrucella IgE forms part of the immune response in brucellosis. This isotype is found with equal frequency in patients with acute and with chronic disease and is not useful in differentiating between the two stages of the disease.

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