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Cryoglobulins in Behçet's syndrome and recurrent oral ulceration: assay by Laser nephelometry

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SUMMARY

The presence of cryoglobulins was investigated in ninety patients with recurrent oral ulcers (ROU) and sixty-one patients with Behçet's syndrome (BS). The immunodiffusion method was compared with Laser nephelometry for the analysis of IgG, IgM, IgA and C3 in cryoglobulins. Although the two methods of assessment showed a very significant agreement, Laser nephelometry was more sensitive than the double diffusion precipitation method and was used for quantitative analysis of cryoglobulins.

The prevalence of any type of cryoglobulin was 64% in ROU and 75% in BS, as compared with controls (15%). In ROU significant levels of IgA were found in minor ($P = 0{\cdot}0196$) and major ($P = 0{\cdot}0114$) aphthous ulcers and to a lesser extent in herpetiform ulcers ($P = 0{\cdot}0624$). Among the four types of BS significant increases in C3 were found in the arthritic type ($P = 0{\cdot}0068$) and ocular type ($P = 0{\cdot}0275$), whereas IgM ($P = 0{\cdot}0031$) and IgG ($P = 0{\cdot}0369$) were increased only in the muco-cutaneous type. Sequential studies showed that disease remissions or exacerbations were correlated with a decrease or increase in IgM or IgG classes of cryoglobulins. However, the converse was found with IgA which may inhibit some functions of polymorphonuclear leucocytes, and this may be responsible for the failure to remove damaging IgG, IgM and C3 complexes from the circulation.

INTRODUCTION

There is a lack of understanding of the pattern of localization of disease. The relationship between recurrent oral ulcers (ROU) and Behçet's syndrome (BS) offers a model for investigating the factors that may be involved in the spectrum of localization of disease (Fig. 1). At the most common end of the spectrum are the mucosal lesions of ROU, followed by oro-genital and in some patients skin lesions of the muco-cutaneous type of BS. The latter can be associated with arthritic, neurological or ocular lesions which occupy the opposite or least common end of the disease spectrum. There is some evidence that this spectrum might have an immunogenetic basis, since the oral and muco-cutaneous lesions are significantly associated with HLA-B12, the arthritic type with HLA-B27 and the ocular type of BS with HLA-B5 (Lehner *et al.*, 1979). These findings suggested the possibility that immune factors might be responsible for disease localization.

Indeed, immune complexes have been found both in patients with ROU and BS, though the prevalence was higher in the latter (Williams & Lehner, 1977; Levinski & Lehner, 1978; Gupta *et al.*, 1978). The relevance of immune complexes to these diseases was established by sequential studies of the IgG class of immune complexes which was closely associated with the disease activity (Levinsky & Lehner, 1978). The involvement of muco-cutaneous surfaces, eyes and joints suggested that the class of

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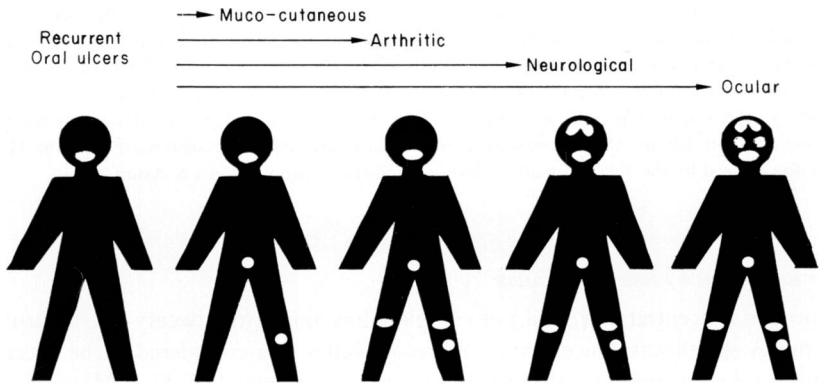

FIG. 1. Spectrum of tissues involved in the four types of Behçet's syndrome and recurrent oral ulcers.

IC might be of some significance, yet the method used was limited to detection of only the IgG class of immune complexes.

In order to overcome this limitation we have studied cold precipitable immune complexes (cryoglobulins) which are found in immune complex disease in man (Brouet *et al.*, 1974; Winfield, Koffler & Kunkel, 1975). The cryoglobulins can be assayed for their different immunoglobulin classes and complement components. The aims of this investigation were to find out if the disease spectrum of tissue involvement can be differentiated by cryoglobulins of different immunoglobulin classes and complement components.

PATIENTS AND METHODS

Cryoglobulins were assayed in 151 patients, consisting of sixty-one patients with BS and ninety with ROU. Cryoglobulins were also assayed in the sera of twenty-one matched control subjects. Patients with ROU were divided into three types, using the criteria defined previously (Lehner, 1968), fifty-five patients had minor aphthous ulcers (MiAU), twenty had major aphthous ulcers (MjAU), and fifteen had herpetiform ulcers (HU). Patients with BS (Fig. 1) were subdivided according to their tissue involvement into four types (Lehner *et al.*, 1979). (a) Muco-cutaneous type, with oro-genital and with or without skin manifestations (fifteen patients). (b) Arthritic type with joint involvement and two or more of the muco-cutaneous lesions (fifteen patients). (c) Neurological type with certain brain disorders and some or all lesions found in the muco-cutaneous and arthritic types (eight patients). (d) Ocular type with uveitis in addition to some or all lesions found in the mucocutaneous, arthritic and neurological types (twenty-three patients). Sequential examinations of cryoglobulins during remission and exacerbation were carried out in each type of ROU and BS. Each ulcer was given an index of 1 and any cutaneous, arthritic or ocular manifestation was each given an index of 1; healing or clearance was given an index of 0·5 and absence of any clinical manifestation was denoted by 0. The individual indices were added up to give the 'clinical index' of disease activity.

Preparation of cryoglobulins. Samples of 20 ml of blood were allowed to clot at 37°C for 4 hr and the serum was separated by centrifugation at 3000 *g* for 15 min at 37°C. The serum was kept at 4°C for 7 days and any cryoprecipitate was separated by centrifugation at 18,000 *g* for 30 min at 4°C and then washed six times in phosphate buffered saline (PBS) at 4°C. The precipitate was dissolved in 0·3 ml of 0·3 M sodium chloride at 37°C, then 0·3 ml of distilled water was added and kept at room temperature until use.

Detection of cryoglobulins by immunodiffusion. The presence of IgG, IgA, IgM, C1q, C3, Factor B, C9, fibrinogen and C-reactive protein (CRP) were tested in all sera by double diffusion against the corresponding antisera at 37°C (Behringwerke, Hounslow, UK). The protein concentration of the cryoglobulin solution was measured by the Lowry method (mg/100 ml of original serum).

Assay of cryoglobulins by Laser nephelometry. The Hyland Laser nephelometer was used to determine the concentrations of IgG, IgM, IgA, C3 and albumin in 106 cryoglobulins, eighty-five specimens from patients as shown in Table 3 and twenty from matched controls. Aliquots of six standard samples (supplied by Hyland) and test samples were pipetted into saline rinsed nephelometer cuvettes; 25 μl for the assay of IgG or IgA, 100 μl for IgM or C3 and 5 μl for the assay of albumin. These samples were then mixed with the antisera diluted with filtered blank diluent (Hyland Reagents) and incubated for 2 hr at room temperature, except the albumin assay which was incubated for 30–45 min. The instrument was set to zero by using the diluent (buffer blank). The range was then set using the highest standard used. The relative light scattering (RLS) of the antibody solution (antibody blank) was measured and held electronically by the nephelometer and then the test solution

(test blank) was measured. The test was then read and the final RLS displayed was that of the actual test, corrected for both the antibody and each test blank RLS. A standard curve was plotted with the known antigen concentrations against the RLS and the results of the test solutions were then read. The results were then expressed as mg/100 ml of the original serum.

Assay of C4, C3 and total haemolytic complement. Serum levels of C4 and C3 were measured by radial immunodiffusion (Fahey & McKelvey, 1965) against specific antisera (Behringwerke, Hounslow, UK) in sixty-seven patients with ROU and forty-seven patients with BS and the results were expressed as a percentage of pooled normal serum. Haemolytic complement (CH50) was assayed by the 50% haemolytic dose method (Lachmann, Hobart & Aston, 1973).

RESULTS

Detection of cryoglobulins by immunodiffusion

The mean protein concentration (±s.d.) of cryoglobulins among the twenty-one controls was 3·98± 7·18 mg/100 ml. A significant concentration of cryoglobulin was considered to be greater than 18·3 mg/100 ml (mean+2 s.d.) and this was not found in any of the controls. IgG, IgM and IgA were found separately in ROU and BS, or as mixed cryoglobulins of IgG and IgM or all three together; C3 was found only in one patient. A significant increase of cryoglobulins in ROU as compared with normal controls was found with the three immunoglobulins separately or in combination with or without C3 (Table 1). Analysis of the three types of ROU showed the highest prevalence of cryoglobulins in MjAU, less in HU and least in MiAU (Table 2).

TABLE 1. Analysis of cryoglobulins* in patients with recurrent oral ulcers (ROU) and sixty-one patients with Behçet's syndrome (BS) by the immunodiffusion method

Ig/C	Number of controls n = 21	Number of ROU (%) n = 90	ROU *vs* controls χ^2	(*P*)	Number of BS (%) n = 61	BS *vs* controls χ^2	(*P*)
IgG	0	16 (18)	4·3621	(< 0·05)	26 (43)	13·1066	(< 0·001)
IgM	0	21 (23)	6·0433	(< 0·02)	26 (43)	13·1066	(< 0·001)
IgA	0	12 (13)	n.s.		16 (26)	6·8435	(< 0·01)
C3	0	1 (1)	n.s.		3 (5)	n.s.	
1-3 Ig	0	23 (26)	6·7693	(< 0·01)	26 (43)	13·1066	(< 0·001)
1-3 Ig ±C3	0	24 (27)	7·1448	(< 0·01)	29 (48)	15·4463	(< 0·001)
C1q	0	9 (10)	n.s.		9 (15)	n.s.	

* Cryoglobulins showing a protein concentration greater than 18·3 mg/100 ml which was the mean+2 s.d. controls. n.s. = Not significant.

In BS all types of cryoglobulins were significantly increased as compared with the controls, except for C3 (Table 1). Analysis of cryoglobulins in ROU as compared with BS showed a significantly increased prevalence in BS of IgG (χ^2 = 11·1778, P<0·001), IgA (χ^2 = 4·003, P<0·05), IgM (χ^2 = 6·3105, P<0·02), 1-3 Ig (χ^2 = 4·8316, P<0·05) and 1-3 Ig±C3 (χ^2 = 6·9546, P<0·01).

Among the four types of BS the highest prevalence of cryoglobulins in any of the Ig classes was found in the muco-cutaneous type; nine of the fifteen patients (60%) showed 1, 2 or 3 Ig±C3 (Table 2). The other types of BS also showed cryoglobulins, in a decreasing order of prevalence as follows: ocular, neurological and arthritic types. C1q was found in 12% to 25% of all groups of patients, except for MiAU in which C1q was found in only 5% (Table 2). CRP and C9 were not detected, C4 was found only in one cryoglobulin and fibrinogen in two cryoglobulins.

Assay of cryoglobulins by Laser nephelometry

Values of cryoglobulins greater than the mean+2 s.d. of the controls were considered to be significant and these were 0·13 mg/100 ml for IgG, 0·56 mg/100 ml for IgM, 0·2 mg/100 ml for IgA and 0·1 mg/100

TABLE 2. Number (%) of components of cryoglobulins* in the three types of patients with recurrent oral ulcers (ROU) and four types of patients with Behçet's syndrome (BS) assayed by the immunodiffusion method

	Type and number of ROU				Type and number of BS			
	Controls	MiAU†	MjAU‡	HU§	Mucocutaneous	Arthritic	Neurological	Ocular
Ig/C	21	55	20	15	15	14	8	24
IgG	0	7 (13)	6 (30)**	3 (20)	8 (53)††	5 (36)**	3 (38)	10 (42)††
IgM	0	7 (13)	11 (55)‡‡	3 (20)	9 (60)‡‡	5 (36)**	2 (25)	10 (42)††
IgA	0	5 (9)	3 (15)	4 (27)¶	4 (27)¶	4 (28)¶	2 (25)	6 (25)¶
C3	0	0	1 (5)	0	1 (7)	1 (7)	0	1 (4)
1 to 3 Ig	0	9 (16)	10 (50)‡‡	4 (27)¶	8 (53)‡‡	4 (28)¶	3 (38)¶	11 (46)‡‡
1 to 3 Ig± C3	0	9 (16)	11 (55)‡‡	4 (27)¶	9 (60)‡‡	5 (36)**	3 (38)¶	12 (50)‡‡
C1q	0	3 (5)	4 (20)	2 (13)	2 (13)	2 (14)	2 (25)	3 (12)

* Cryoglobulins showing a protein concentration greater than 18·3 mg/100 ml which was the mean+2 s.d. † Minor aphthous ulcers. ‡ Major aphthous ulcers. § Herpetiform ulcers. ¶ $P< 0·05$.** $P< 0·02$. ‡‡ $P< 0·0005$. †† $P< 0·002$.

TABLE 3. Analysis of cryoglobulins in forty-five patients with recurrent oral ulcers (ROU) and forty patients with Behçet's syndrome (BS) by Laser nephelometry

Ig/C	Number of controls (%) n = 20	Number of ROU (%) n = 45	ROU *vs* controls *P**	Number of BS (%) n = 40	BS *vs* controls *P**
IgG	1 (5)	11 (24)	n.s.	15 (38)	0·0115
IgM	1 (5)	10 (22)	n.s.	12 (20)	0·0479
IgA	1 (5)	18 (40)	0·0059	13 (32)	0·0304
C3	0	9 (20)	n.s.	11 (28)	0·0134
1–3 Ig	3 (15)	20 (44)	0·0389	19 (48)	0·0252
1–3 Ig±C3	3 (15)	29 (64)	0·0004	30 (75)	0·00002

* Fisher's exact test.
n.s. = Not significant.

ml for C3. Fisher's exact test was used for the analysis of all groups. A significant increase in ROU as compared with normal controls was found with IgA ($P = 0{\cdot}0059$), 1–3 Ig ($P = 0{\cdot}0389$) and with 1–3 Ig ±C3 ($P = 0{\cdot}0004$; Table 3). MiAU and MjAU showed a significantly increased prevalence with IgA ($P = <0{\cdot}05, <0{\cdot}02$) and in the combined group of 1–3 Ig±C3 ($P = <0{\cdot}02, <0{\cdot}001$; Table 4). C3 was increased significantly only in MjAU ($P = 0{\cdot}0185$). Cryoglobulins were also increased in HU, but the 5% value of significance was reached only with 1–3 Ig±C3 ($P = 0{\cdot}0367$).

In BS all components of cryoglobulins were significantly increased (Table 3). Among the four types of BS, C3 was significantly increased in the arthritic and ocular types, IgM and IgG in the M-C and IgA in none, whereas 1–3 Ig±C3 in all four types (Table 4).

Sequential examination over 3–6 months during remission or exacerbation of the disease revealed quantitative changes in the cryoglobulins, as well as changes in isotype (Figs 2 and 3). A decrease in the clinical index was in most instances associated with a corresponding decrease in the concentrations of IgM and/or IgG. Exacerbation in the ocular type of BS was associated with a significant increase in concentration of IgM, and the arthritic type there was a particularly large increase in IgG. However, IgA showed the reverse trend, in that a decrease in the clinical index was associated with an increase in the concentration of IgA in four out of five patients (Fig. 3). Both the exacerbations were associated with a significant decrease in IgA. A consistent relationship between the concentration of C3 and the clinical remission or exacerbation was not evident.

Comparison of the detection of immunoglobulins and C3 by the immunodiffusion and Laser nephelometry methods

A very significant agreement was found between the two methods for IgG ($\chi^2 = 40{\cdot}59$, $P<0{\cdot}0001$), IgM ($\chi^2 = 50{\cdot}50$, $P<0{\cdot}0001$) and IgA ($\chi^2 = 13{\cdot}93$, $P<0{\cdot}001$). There were only four cryoglobulins with C3 detectable by immunodiffusion, so that these results are not shown. Forty-two out of 161 cryoglobulins showed measurable C3 by Laser nephelometry and twenty-six of these had significant values (>0·1 mg/100 ml).

Assay of C4, C3 and total haemolytic complement

There was no significant difference in serum C4, C3 and CH50 concentrations between the various groups of patients and those found in normal subjects. C4 and C3 were not depressed in sixty-seven patients with ROU or in forty-seven patients with BS. CH50 was depressed below 60% of normal in three out of thirty-two patients tested with ROU and in one out of thirty patients with BS. This rather small number of depressed complement levels was not significant. A somewhat larger number showed increased levels of C3 (>160%); seven out of sixty-seven patients with ROU and eight out of forty-seven patients with BS. Furthermore, four out of thirty-one sera from BS tested showed increased levels (>200%) of C4 but none of the sera from ROU showed this. CH50 was not increased in any of the tested sera.

TABLE 4. Number (%) of cryoglobulin components in the three types of recurrent oral ulcers and four types of Behçet's syndrome assayed by Laser nephelometry

	Recurrent oral ulcers				Behcet's syndrome			
Ig/C	Controls $n = 20$	MiAU¶ $n = 20$	MjAU** $n = 15$	HU†† $n = 10$	Muco-cutaneous $n = 12$	Arthritic $n = 8$	Neurological $n = 8$	Ocular $n = 12$
IgG	1 (5)	3 (15)	4 (27)	4 (40)	5 (42)*	3 (38)	3 (38)	4 (33)
IgM	1 (5)	3 (15)	5 (33)	2 (20)	7 (58)‡	1 (12)	2 (25)	2 (17)
IgA	1 (5)	7 (35)*	7 (47)†	4 (40)	3 (25)	3 (38)	3 (38)	4 (33)
C3	0	2 (10)	5 (33)	2 (20)	2 (17)	4 (50)‡	1 (12)	4 (33)*
1 2 or 3 Ig	3 (15)	9 (45)	7 (47)	4 (40)	6 (50)	1 (12)	5 (62)*	7 (58)*
1–3 Ig± C3	3 (15)	11 (55)†	12 (80)§	6 (60)*	8 (67)*	5 (62)*	6 (75)†	11 (92)§

Analysis by Fisher's exact test between the disease groups and controls: * $P < 0{\cdot}05$; † $P < 0{\cdot}02$; ‡ $P < 0{\cdot}01$; § $P < 0{\cdot}001$; ¶ minor aphthous ulcers; ** major aphthous ulcers; †† herpetiform ulcers.

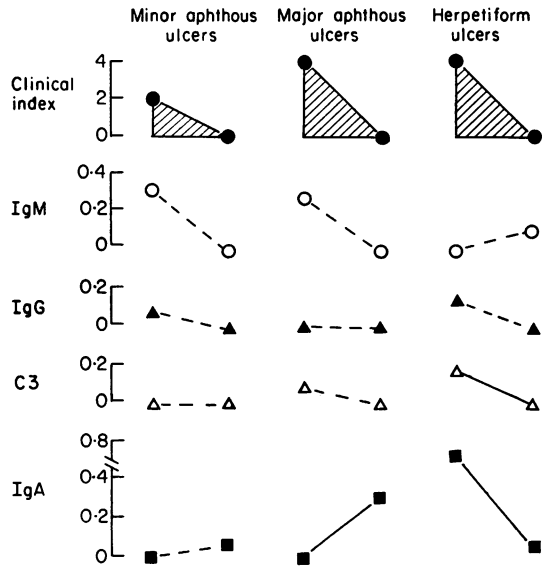

FIG. 2. Sequential assay of cryoglobulins in the three types of recurrent oral ulcers. (—— Significant change, - - - not significant change.)

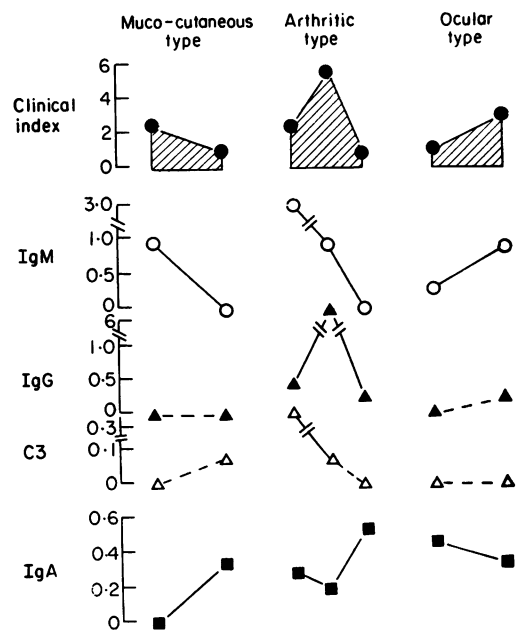

FIG. 3. Sequential assay of cryoglobulins in three types of Behçet's syndrome. (—— Significant change, - - - not significant change.)

DISCUSSION

Significant cryoglobulins of the IgG, IgM or IgA class, with or without C3, were found in ROU ($P<0{\cdot}01$–$0{\cdot}001$) and BS ($P<0{\cdot}001$–$<0{\cdot}0001$) irrespective of the method of assessment. The prevalence of any type of cryoglobulin was 27% in ROU and 48% in BS, by the double diffusion precipitation method and these figures increased to 64% in ROU and 75% in BS by Laser nephelometry. The data are consistent with the concept that cryoglobulins are cold precipitable immune complexes (Brouet

et al., 1974), as the prevalence of cryoglobulins was broadly comparable with that of soluble immune complexes (40% in ROU and 60% in BS; Levinsky & Lehner, 1978).

A comparative analysis of the two methods of assessment of the three immunoglobulins classes of cryoglobulins showed a highly significant agreement. However, as expected the Laser nephelometry method was more sensitive than the immunodiffusion method. Samples were negative by the immunodiffusion method but positive by Laser nephelometry with C3 in 26%, IgG in 27%, IgA in 30% and IgM in 38%; the converse was true only in 0–6% of the samples of about 161 cryoglobulins tested. The results of the two methods of assay, however, are not strictly comparable, because quantitative assessment of each of the three Ig and C3 was carried out only by Laser nephelometry. The double diffusion precipitation method allowed only qualitative assessment for the presence or absence of a particular component. Furthermore, a protein level of 18·3 mg/100 ml in cyroglobulins was used to differentiate between those found in controls and patients. This concentration accounts for all the proteins, including any antigen, so that its application to any specific immunoglobulin or C3 must be considered only as an arbitrary level. For these reasons the data derived by Laser nephelometry are more accurate with regard to individual components.

A comparison of cryoglobulins between the three types of ROU failed to reveal significant differences, though the prevalance of IgG, IgM, IgA and C3 was greater in MjAU than in MiAU (Table 4). Since oral ulceration might be considered as a common denominator throughout the spectrum of ROU and BS, it is not surprising that IgA is increased in all of them, with the highest prevalance in MjAU which comprises the most severe mucosal lesion (Table 4). Similar results were found with the IgA class of immune complexes, determined by an agglutination inhibition method, except that HU failed to yield a significant prevalence of IgA immune complexes (Levinsky, Paganelli & Lehner, 1979).

Significant increases in the various components of cryoglobulins were found in the four types of BS. IgG and IgM were significantly increased only in the M-C type (Table 4). C3 showed the highest frequency in the arthritic (50%; $P<0{\cdot}01$) and ocular types (33%; $P<0{\cdot}05$; Table 4) and the presence of C3 seems to be the best laboratory discriminator in the differential diagnosis of the arthritic and ocular types from the M-C and neurological types of BS as well as from MiAU. It is worth noting that the ocular type of BS showed the highest prevalence (92%) of any type of cryoglobulin. The increase of C3 in BS might be particularly important in a multifocal disease, as there is some evidence that complement is necessary for transport of immune complexes (Embling *et al.*, 1978). However, no significant differences in the levels of C3, C4 or CH50 were found in sera of patients with BS, although a fall in C4, C3 and C2 has been detected on sequential analysis of uveitis (Shimada *et al.*, 1974). It might be of interest that a significant increase was found with serum C3 in 17% and C4 in 13% of patients with BS and this is consistent with the much greater increase in the concentration of C9 found in sera from patients with BS (Adinolfi & Lehner, 1976).

Cryoglobulins can play a pathogenic role in disease by complement activation (Muller, Rother & Westerhausen, 1976), platelet aggregation (Cortellaro *et al.*, 1975) and cytotoxic activity (Winfield *et al.*, 1975). Injection of antigen leads to formation of cryoglobulins in parallel with immune complexes, and the injected antigen and antibody have been detected in these cryoglobulins Griswold *et al.*, 1974; Moroz, Comerford & Guttman, 1975). The functional role of cryoglobulins found in ROU and BS is not clear, but sequential studies of remissions and exacerbations of the disease are generally correlated with a decrease or increase in IgM or IgG (Figs. 2 and 3). A potentially important finding was the converse relationship between IgA and disease activity. Since IgA cryoglobulins show a significant negative correlation with chemotaxis (Abdulla & Lehner, 1979), it is suggested that complexes in ROU and BS might cause steric interference with the chemotactic receptors on PMNL by attachment to the IgA Fc receptors, as was proposed for myeloma IgA (Van Epps & Williams, 1976). This might also lead to a depressed phagocytic function of the affected PMNL, as has been demonstrated in cells from patients with BS (Wilton & Lehner, 1979). A defect in chemotaxis and phagocytosis induced by IgA immune complexes may be responsible for the failure to remove IgG, IgM and C3 complexes and this may lead to complement dependent damage at the site of the lesion.

The hitherto almost exclusive clinically based diagnosis of BS can be strengthened by HLA typing

(Ohno *et al.*, 1975; Lehner *et al.*, 1979), assay for immune complexes (Levinsky & Lehner, 1978), C9 and C-reactive protein (Adinolfi & Lehner, 1976). These tests might also be useful in the prognosis of the rare but potentially serious change from focal oral ulceration to the multifocal BS. Assay of cold precipitable complexes might be more informative than those for immune complexes, as the immunoglobulin class and complement components are determined in the same test.

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REFERENCES

ABDULLA, J. & LEHNER, T. (1979) The effect of immune complexes on chemotaxis in Behçet's syndrome and recurrent ulcers. *Behçet's Syndrome* (ed. by T. Lehner and C. G. Barnes). Academic Press, London (in press).

ADINOLFI, M. & LEHNER, T. (1976) Acute phase proteins and C9 in patients with Behçet's syndrome and aphthous ulcers. *Clin. exp. Immunol.* **25**, 36.

BROUET, I.C., CLAUVEL, I.P., DANON, F., KLEIN, M. & SELLIGMANN, M. (1974) Biologic and clinical significance of cryoglobulins. *Amer. J. Med.* **57**, 775.

CORTELLARO, M., LAMBERTENGHI-DILILIERS, G., CONFRANCESCO, E., POGLIANI, E., POZZOLI, E., IMBASCIATI, E. & PRAGA, C. (1975) Human platelet aggregation by mixed cryoglobulins. *Acta Haemat.* **54**, 36.

EMBLING, P.H., EVANS, H., GUTTIEREZ, C., HOLBOROW, E.J., JOHNS, P., JOHNSON, P.M., PAPAMICHAIL, M. & STANWORTH, D.R. (1978) The structural requirements for immunoglobulin aggregates to localize in germinal centres. *Immunology* **34**, 781.

FAHEY, J.L. & MCKELVEY, E.M. (1965) Quantitative determination of serum immunoglobulins in antibody-agar plates. *J. Immunol.* **94**, 84.

GRISWOLD, W.R., KOSS, M., CHERNACK, W.J., WEIL, R., SUTPHEN, J. & MCINTOSH, R.M. (1974) Glomerular localization of antigen and antibody in rabbits following intravenous administration of serum cryoproteins from homologous animals with acute serum sickness. *Proc. Soc. Exp. Biol. Med.* **145**, 117.

GUPTA, R.C., O'DUFFY, J.D., MCDUFFIE, F.C., MEURER, M. & JORDON, R.E. (1978) Circulating immune complexes in the blood of patients with Behçet's syndrome. *Clin. exp. Immunol.* **34**, 213.

LACHMANN, P.J., HOBART, M.J. & ASTON, W.P. (1973) Complement technology. *Handbook of Experimental Immunology* (ed. by D.M. Weir), p. 5. Blackwell Scientific Publications, Oxford.

LEHNER, T. (1968) Autoimmunity in oral diseases with special reference to recurrent oral ulceration. *Proc. Roy. Soc. Med.* **61**, 515.

LEHNER, T., BATCHELOR, J.R., CHALLACOMBE, S.J. & KENNEDY, L. (1979) An immunogenetic basis for the tissue involvement in Behçet's syndrome. *Immunology*, **37**, 895.

LEVINSKY, R.J. & LEHNER, T. (1978) Circulating soluble immune complexes in recurrent oral ulceration and Behcet's syndrome. *Clin. exp. Immunol.* **32**, 193.

LEVINSKY, R.J., PAGANELLI, R. & LEHNER, T. (1979) Immune complexes and their characterization in Behçet's syndrome and recurrent oral ulcers. *Behçet's syndrome.* (Ed. by T. Lehner and C.G. Barnes). Academic Press, London (in press).

MOROZ, L.A., COMERFORD, T.A. & GUTTMAN, R.D. (1975) IgG-IgA cryoglobulinaemia in human serum sickness. Evidence for Equine IgG in the cryoprecipitate. *Int. Arch. Allergy Appl. Med.* **48**, 756.

MULLER, S., ROTHER, U. & WESTERHAUSEN, M. (1976) Evidence for a pathogenic role of an IgG (KAPPA) cryoglobulin in cutaneous vasculitis. *Clin. exp. Immunol.* **23**, 233.

OHNO, S., NAKAYAMA, E., SUGIURA, S., ITAKURA, K., AOKI, K. & AIZAWA, M. (1975) Specific histocompatibility antigens associated with Behçet's syndrome. *Am. J. Ophthalmol.* **80**, 636.

SHIMADA, H., KOGURE, M., KAWASHIM, T. & NISHIOKA, K. (1974) Reduction of complement in Behçet's disease and drug allergy. *Med. Biol.* **52**, 234.

VAN EPPS, D.E. & WILLIAMS, R.C. (1976) Suppression of leukocyte chemotaxis by human IgA myeloma components. *J. exp. Med.* **144**, 1227.

WILLIAMS, B.D. & LEHNER, T. (1977) Immune complexes in Behçet's syndrome and recurrent oral ulceration. *Brit. Med. J.* **1**, 1387.

WINFIELD, J.B, KOFFLER, D. & KUNKEL, H.G. (1975) Specific concentration of polynucleotide immune complexes in the cryoprecipitates of patients with systemic lupus erythematosus. *J. Clin. Invest.* **56**, 563.

WILTON, J.M.A. & LEHNER, T. (1979) Defective polymorphonuclear leukocyte phagocytosis in patients with Behçet's syndrome and recurrent oral ulcers. *Behçet's Syndrome.* (Ed. by T. Lehner and C.G.Barnes). Academic Press (in press).