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Anti-DNA antibodies and circulating immune complexes (C1q-IgG) in recurrent aphtous stomatitis

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SUMMARY

Anti-DNA antibodies and circulating immune complexes (C1q-IgG) in sera from 50 patients with minor aphtae at the moment of study and 50 healthy controls, were determined. The obtained results were correlated with chronological and clinical parameters of the disease. Our findings show a greater number of patients than controls with higher values of anti-DNA antibodies. The time from the last active stage of the disease conditioned the anti-DNA antibody levels ($p < 0.001$). However, no significant differences among the number of recurrences in a year or the number of lesions in a ulcerative stage and the anti-DNA antibody concentrations were observed.

KEY WORDS:

Anti-DNA antibodies, circulating immune complexes C1q-IgG, recurrent aphtous stomatitis

RÉSUMÉ

Cette étude a pour but de déterminer les anticorps anti-ADN et les complexes immuns circulants C1q-IgG dans le sérum de 50 patients qui présentaient des aphtes mineurs au moment de l'étude et chez 50 contrôles. Les résultats obtenus sont en rapport avec les paramètres cliniques et chronologiques de la maladie. Nous avons trouvé des niveaux plus élevés d'anticorps anti-ADN chez les patients avec des aphtes mineurs, en comparaison avec les contrôles. Il existait une corrélation entre ces niveaux d'anticorps anti-ADN et le temps écoulé depuis le dernier épisode de la maladie ($p < 0.001$). Il n'existait cependant pas de différences significatives entre les concentrations anti-ADN ni en ce qui concerne le nombre de récidives ni le nombre de lésions durant l'épisode.

MOTS-CLÉS:

Anticorps anti-DNA, complexes immuns circulants C1q-IgG, stomatite aphteuse récidivante.

INTRODUCTION

Recurrent aphtous stomatitis (RAS) is one of the most common oral disorder (Cawson and Eveson, 1987). RAS affects, approximately about 2% to 60% of the population, depending on the group studied (Scully and Porter, 1989). Despite its frequency, the processes involved in its development still remain unresolved. Several hypotheses have been proposed as possible causes: viral (Pedersen, 1991), bacterial (Greenspan and Shillitoe, 1984), genetic-basis (Albanidou-Farmaki et al., 1990) and, most recently, a immunologic basis has been proposed (Bergamini et al., 1990; Landesberg et al., 1990). Natural killer cells (NK) could be related to several active stages of the disease, as some studies (Sun et al., 1991) have proved, showing, at early stages, a increase of ADCC (antibody-dependent cellular cytotoxicity) due to: an increase in either the number and/or the efficiency of the effector cells; an increment in the similarities of the Fc receptors. Other studies (Pedersen et al., 1991) have pointed out changes in T helper lymphocytes (CD4+) and T suppressor cells (CD8+) rates during the disease spread. The increased of T helper lymphocytes and/or the reduced of CD8+ cell counts could be the basis of an immune response, leading to an increment of the antibody producing cells against epithelial antigens. However, the processes involved in these immunologic findings has not been resolved. Some other investigations (Sun et al., 1986) showed that circulating immune complexes carry out a important role in the multifocal appearance within systemic processes characterized by aphtae of the Behçet's syndrome (BS).

The purpose of the present study was to investigate further anti-DNA antibody concentrations influence as the possible origin of this disease, as well as the possible role of circulating immune complexes (C1q-IgG). The findings were related to some chronological aspects of the disease, such as: time from the last active stage, duration of each active stage and number of recurrences in a year.

MATERIALS AND METHODS

The population of our study consisted of 100 people (38 men and 62 women). There were 50 patients (aphtous group) who showed aphtae at the moment of study; and 50 healthy controls (control group) who never had the disease. 5 ml of peripheral blood samples were collected from both groups. Serum was separated and stored at −20°C. Anti-DNA antibodies and circulating immune complexes (C1q-IgG) (CIC) detection was determined using commercial kits consistent on indirect immune assay techniques in microwell strips coated with double strain DNA (ELIAS Medizintechnik™ GmbH) and microwell strips coated with monoclonal antibody (murine) to human C1q (IMMUSTRIP C1q Immunomedics ™). Photometric quantitation was made using a microwell strip reader, set a 492 nm. The results of anti-DNA antibody were expressed in 6 concentration levels according to the standard values provided: 0, 0-12.5, 12.5-25, 25-50, 50-100 and 100-200 IU/ml respectively and those of CIC in 3 levels according to the standard values provided: 0-18 micrograms/ml, 18-48 micrograms/ml and 48-140 micrograms/ml. Each patient was clinically evaluated including chronological and clinical data of the disease, disregarding those patients who had previous autoimmune disease manifestations such as rheumatoid arthritis or systemic lupus erythematosus. The results were processed by SPSS-PC+ statistical programme, using the «Student t-test» for quantitative variables and the «Chi-square test» for qualitative variables. P-values below 0.05 were considered significant.

RESULTS

All the patients included in our study had minor forms of recurrent aphtous stomatitis (RAS). However, in some cases, the coalescence of several minor aphtae gave the appearance of major aphtae.

Evaluating of anti-DNA antibodies showed that in the aphtous group, 16 (32%) had no anti-DNA antibodies (0 IU/ml), 9 (18%) presented concentrations between 0-12.5 IU/ml, 10 (20%) showed concentrations between 12.5-25 IU/ml, 3 (6%) had concentrations between 25-50 IU/ml, 6 (12%) presented concentrations between 50-100 IU/ml and 6 (12%) had concentrations between 100-200 IU/ml. In the control group, anti-DNA antibodies were not found in 38 of them (76%), 8 (16%) had concentrations between 0-12.5 IU/ml, 2 subjects (4%) presented concentrations between 12.5-25 IU/ml and other 2 of them (4%) had titers between 100-200 IU/ml.

In Fig. 1 illustrates the distribution of the samples containing anti-DNA antibodies. A highly significant association ($p < 0.001$) between the two variables «presence of aphtae» and «presence of anti-DNA antibodies» was seen.

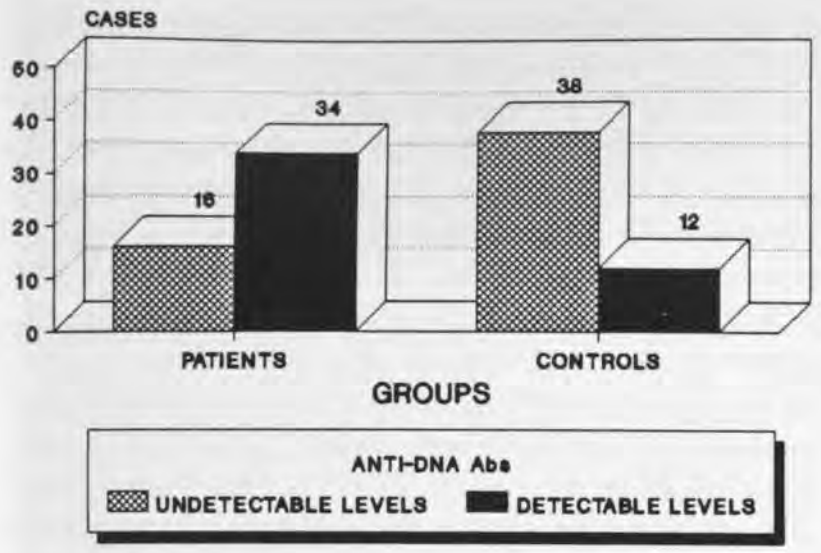

Fig. 1: Determination of anti-DNA antibodies in serum in patients and controls.
Fig. 1: Détermination des anticorps anti-ADN dans le sérum des contrôles et des patients.

31 (62%) out of 50 people from the aphtous group had circulating immune complex concentrations between 0-18 micrograms/ml, 16 patients (32%) presented titers between 18-48 micrograms/ml and 3 subjects (6%) showed concentrations between 48-140 micrograms/ml. In the control group, 42 684%) had circulating immune complex concentrations between 0-18 micrograms/ml, 6 (12%) presented titers between 18-48 micrograms/ml and 2 (4%) showed concentrations between 48-140 micrograms/ml (Fig. 2).

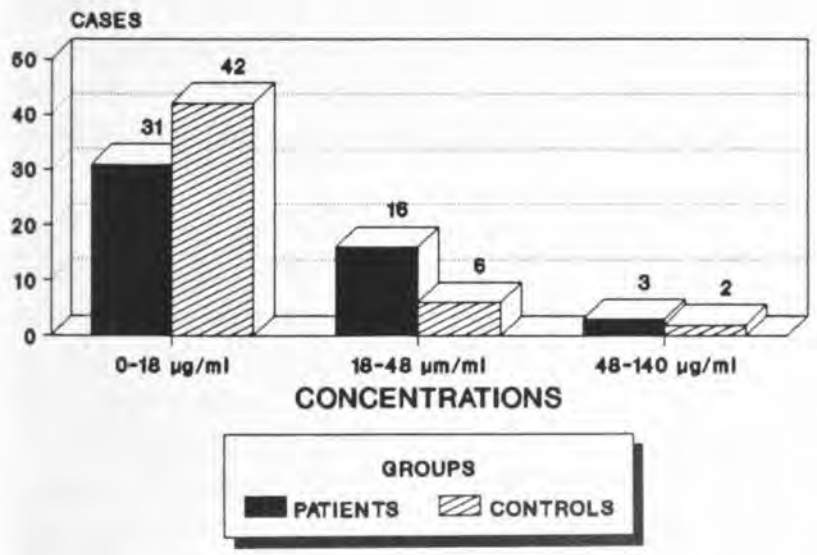

Fig. 2: Circulating immune complex (C1q-IgG) concentrations in the patient group and control group.
Fig. 2: Concentrations de complexes immuns circulants C1q-IgG des contrôles et des patients.

CIC values were higher in the aphtous group than those found in the control group. A significant difference (p=0.0407) was seen between population groups within the concentration levels. Comparison of number of subjects with normal (<18 micrograms/ml) and high (>18 micrograms/ml) CIC values in both groups showed significant differences (p=0.0132).

In order to estimate the possible association among these parameters, the results of both serum detections were related to the chronological data of the disease.

In Fig. 3 the abscissa axis shows the time from the last active stage; on the ordinate axis we can appreciate the percentage of subjects with detectable and non detectable anti-DNA antibody titers. A highly significant difference (p<0.001) between both populations was found.

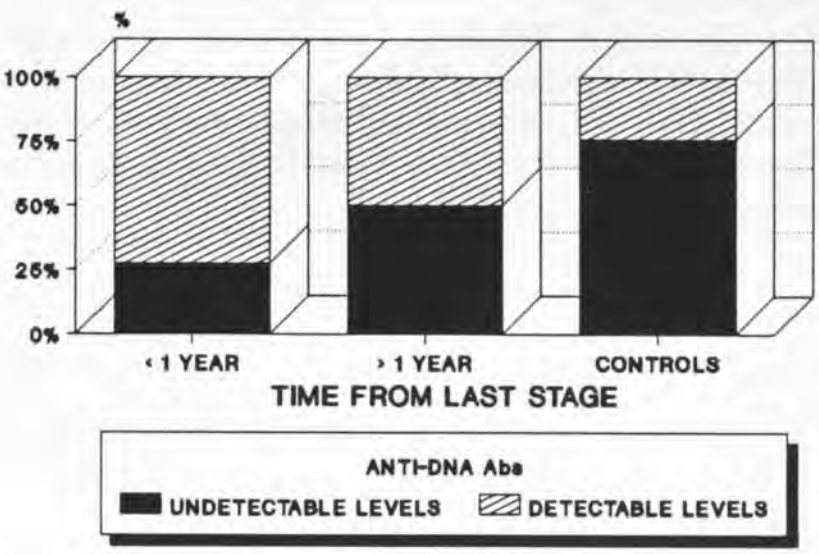

Fig. 3: Relation between the time from the last active stage of RAS and anti-DNA antibody levels.
Fig. 3: Relation entre le temps écoulé depuis le dernier épisode de la stomatite aphteuse récidivante et les niveaux d'anticorps anti-ADN.

In contrast, there was no significant association when comparing the presence of anti-DNA antibodies and the recurrence periods of the disease in the last year. No significant association between duration of the RAS episode (expressed in 3 intervals: 1-7 days, 8-14 days and 15-21 days) and anti-DNA antibodies detection was seen.

The comparison between the circulating immune complexes (C1q-IgG) normal (<18 micrograms/ml) and high levels (>18 micrograms/ml) and the time from the last active stage proved significant (p=0.0459) (Fig. 4).

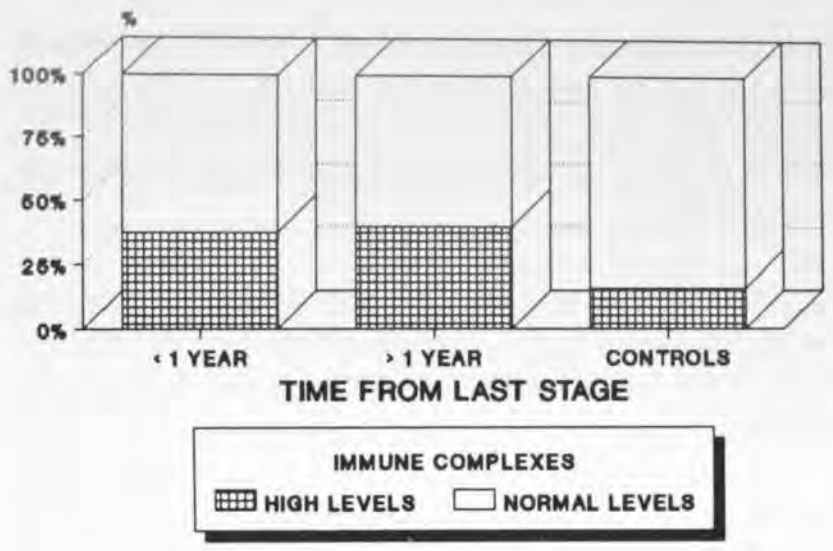

Fig. 4: Relation between the time from the last active stage of RAS and the circulating immune complex (CAq-IgG) levels.
Fig. 4: Relation entre le temps écoulé depuis le dernier épisode de la stomatite aphteuse récidivante et les niveaux de complexes immuns circulants C1q-IgG.

On the other hand, no significant difference ($p=0.5413$) between circulating immune complex concentrations and the annual recurrence or the duration of an active stage of the disease was found in both groups.

DISCUSSION

Autoimmunity as an etiopathogenical factor in RAS was proposed by several researchers (Roitt and Lehner, 1987).

Despite that, the existing bibliography brings not much information of serum anti-DNA antibody determinations using enzymoimmunoanalysis techniques (ELISA) or other immunodiagnostic techniques.

Malstrom et al. (1983) carried out a study on 20 RAS patients in order to determine immunoglobulin levels (Ig), Complement fractions (C) and immunogenetic markers; anti-DNA antibodies were found in 5 patients (25% of the cases).

Later on, Sun and Wu (1989) showed, using indirect immunofluorescence techniques, serum anti-DNA antibodies and antibodies against intercellular substance in a study on 102 patients, from which 21 were RAS patients, 41 controls and 40 had other mucosal diseases. 15 RAS patients had anti-DNA antibodies (71% of the cases). Jaremko et al. (1990) in a study on only 4 patients proved high anti-DNA antibody titers in serum.

In our study, serum anti-DNA antibody concentrations were measured at intervals. However, when considering as a whole those who had anti-DNA antibodies and those who had not, the results were as follows: in the aphtous group, 68% had antibodies whereas in 32% the results were negative. As regards the control group, we had 34% of positive cases and 76% of negative cases, finding highly significant differences ($p<0.001$) between both subsets. The results of our sample agreed with those published by Sun and Wu (1989) though showing differences with those by Malstrom et al. (1983), who found only 25% of positive cases. Nevertheless, the small amount of cases studied by the mentioned researchers (20-21 cases respectively) could be the reason of their different conclusions.

In our study, estimating the circulating immune complexes (C1q-IgG) (CIC) detection in serum, high concentrations of CIC were found in 38% of the aphtous group and 16% of the control group. Furthermore, 62% of the aphtous group and 84% of the control group had normal levels of CIC.

Palmqvist (1976), determined serum levels of C1, C3 and C4, finding no increase in the immune complexes in the RAS populations. Their showed no significant differences between RAS patient and control concentrations. However, Hornstein and Djawari (1980) found high OgG immune complex levels in RAS and Behçet's syndrome BS) patients. In 1987, in a study carried out on 52 patients (21 RAS patients and 31 who never had the disease) Bagg et al. (1987) found only one patient from the disease group (4.76%) with high immune complexes values.

In a sample of 36 patients, 17 with BS, 11 with RAS and 8 controls, Williams and Lehner (1977) obtained the following results: 33% of BS patients and 28% of RAS cases showed high levels of CIC, whereas no controls had high levels. This author also compared CIC titers to three clinical RAS groups. There are higher immune complex levels in herpes-like ulcerations group (HU) than in the other two groups: major aphtae (MaRAS) and minor aphtae (MiRAS).

Lehner et al. (1979) studied IgG concentrations, other immunoglobulin and complement fractions in RAS patients, BS patients and controls, using nephelometry. The results pointed out that 64% of RAS patients had increased levels compared to 75% of BS patients and only 15% of the controls.

Levinsky and Lehner (1978) studied IgG circulating immune complexes with agglutination-inhibition tests, finding that 60% of BS patients had high concentration serum whereas 40% of RAS patients showed the same concentration values (in a population consisting of 30 BS patients, 30 RAS patients and 30 controls).

Our results could be in agreement with Savage et al. (1985), Ratis et al. (1991) and Pedersen et al. (1992) studies when regarding to the variation of T helper/T suppressor cell counts throughout the different disease stages. In that sense, an increase in T helper lymphocytes and/or a decrease in T suppressor lymphocytes could be the basis of immune response disturbances determining antibody producing cells proliferation, leading to the circulating immune complexes formation whose levels increased in RAS and BS.

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