Denture stomatitis: Quantification of interleukin-2 production by mononuclear blood cells cultured with *Candida albicans*

Alberto Rodriguez-Archilla, DDS, PhD,[a] Miguel Urquia, MD, PhD,[b] Antonio Cutando, MD, PhD,[c] and Raquel Asencio, BDS[d]

School of Dentistry, University of Granada, Granada, Spain

Denture stomatitis is usually associated with the presence of yeast, particularly *Candida albicans*, and several bacteria. In this study mononuclear blood cells were grown in the presence of *Candida albicans* from a single colony, and interleukin-2 production induced in T lymphocytes was measured. Blood cells were from a population of patients with denture stomatitis and a control group of denture wearers without stomatitis. Induction of interleukin-2 production was correlated with factors that condition denture stomatitis, namely, isolation of *Candida albicans* in selective medium, age of the denture, and diabetes. Concentrations of interleukin-2 in supernatant and serum were also compared. Significant differences in interleukin-2 production were found between patients with denture stomatitis and controls. Statistical analysis demonstrated a significant association between isolation of *Candida albicans* and elevated interleukin-2 production in cultures from patients with and without denture stomatitis. (J PROSTHET DENT 1996;75:426-31.)

Denture stomatitis is the most common oral disorder found in denture wearers. It is usually associated with the presence of yeasts, particularly *Candida albicans*, and several bacteria.[1-4] Previous studies have noted the importance of T lymphocytes in the cell-mediated response to *Candida* microorganisms.[5,6] For antigen recognition by the lymphocyte to occur, the antigen must associate with class II histocompatibility (HLA) molecules on the surface of antigen-presenting cells. The HLA molecules thus act as restrictors of antigen recognition. Once the T lymphocytes have been stimulated, they are transformed and they multiply to produce large numbers of activated cells. This process leads to the appearance of effector and memory T lymphocytes. Studies[7,8] suggested that T cell proliferation is determined by cytokines, including interleukin-2 (IL-2) and interferon.

Wei et al.[9] claimed that IL-2 and interferon production are modified by anti–class I and class II HLA monoclonal antibodies (anti-A, B, C, anti-DRIDP, and DQ). Scarigni et al.[10] suggested that mannose in the *Candida* cell wall or metabolites of mannose inhibit immune system activity against the microorganism. Podzorski et al.[11] documented the immunomodulatory effects of other cell wall polysaccharides and demonstrated that when overgrowth of *Candida* occurred these antigens entered the bloodstream and exerted an immunosuppressive effect, in agreement with earlier findings in our laboratory.[12] In this connection findings by Fischer et al.[13] provided support for the hypothesis of the inhibitory action of mannose or its metabolites. They showed that the immunosuppressive effect of these molecules disappeared with antifungal therapy.

Nevertheless, this fact is controversial. Ausiello et al.[14] studied the expression of cytokine genes in cultures of human peripheral blood mononuclear cells stimulated with mannoprotein constituents (MP) of *C. albicans*, purified protein derivative from *Mycobacterium tuberculosis* and IL-2. Their results showed that MP of *C. albicans* were able to induce IL-2 messenger ribonucleic acid. This induction of cytokine gene expression by MP underlined the importance of cell wall constituents and cytokines in the anticandidal host response.

Studies by Budtz-Jorgensen[15] with different microbial antigens and Damle et al.[16] in patients with candidiasis underlined the importance of the helper/suppressor ratio of T-lymphocyte subpopulations in the immune response. The helper T-lymphocyte subpopulation has been classically considered to be an inducer of cellular response and differentiation because it is induced by the production of IL-2 and other cytokines.[17] IL-2 is known to have a direct effect on antibody-producing clones of B lymphoblasts and activated T-lymphocyte proliferation. IL-2 is also known to induce phagocytic capacity in polymorphonuclear leukocytes and the destruction of target cells by natural killer cells and cytotoxic T lymphocytes.

This study was designed to measure IL-2 production in mononuclear blood cells from patients with clinically detectable denture stomatitis. The cells were grown in vitro in the presence of *C. albicans*, and IL-2 production

Research supported by the Andalusian Regional Government through the Research Team Training Program (Plan de Formacion de Grupos de Investigacion).

[a]Associate Professor, Department of Oral Medicine.
[b]Professor, Department of Oral Medicine.
[c]Professor, Department of Special Care in Dentistry.
[d]Scholar, Department of Oral Medicine.

0022-3913/96/$5.00 + 0. **10/1/70211**

was compared with that of cells from denture wearers without denture stomatitis (control patients). The IL-2 production was related in both populations with the detection of *C. albicans* in selective medium, clinically detectable denture stomatitis lesions according to three clinical Newton's types, and subjective discomfort (pain or burning) reported by patients. The influence of etiologic factors in denture stomatitis on IL-2 production in vitro was also investigated.

MATERIAL AND METHODS

Patients

The study population consisted of 44 complete denture–wearers, of whom 29 had clinically proved denture stomatitis (stomatitis patient group). The remaining patients without denture stomatitis ($n = 15$) served as the control group. Of the 44 patients, there were 20 men and 24 women, and the age range of the population was 35 to 74 years (mean 58.4 years). There were no significant age or sex differences between the stomatitis patient and control groups. Informed consent to participate in the study was obtained from all patients.

Diagnosis

Diagnosis of denture stomatitis was made on the basis of the clinical appearance, classified as localized erythematous, diffuse erythematous, or hyperplastic granular (Newton's types). All the oral examinations were completed by the same examiner.

Protocols

For each patient some local and general factors that might have conditioned denture stomatitis were recorded: diabetes, isolation of *C. albicans* in selective medium, denture age, and oral hygiene habits. The clinical features noted were detectable lesions, subjective symptoms, and degree of affectation of the oral mucosa. *Candida* culture, differential leukocyte count, and enzyme immunoanalysis to determine IL-2 concentrations in serum and supernatant were also performed for each patient. Patients whose leukocyte counts (normal range lymphocytes 20% to 45%, monocytes 2% to 6%, neutrophils 45% to 70%, eosinophils 0% to 3%, and basophils 0% to 2%) were abnormal were excluded from further study. Moreover, patients with nutritional deficiencies, immunosupressive or allergic disturbances, and prolonged treatment with antibiotics or other drugs were also excluded. *C. albicans* was detected by culture in Sabouraud-chloramphenicol-actidione medium (Pasteur) of samples obtained from the hard palate with a sterile cotton-tipped swab.

The samples were incubated at 37° C, and the results were read after 24 to 48 hours. *Candida* was then incubated in pooled sera for 24 hours to detect the formation of typical *C. albicans* hyphae.

A 10 ml venous blood sample was obtained from each participant and divided in two parts. One part was used to separate serum and was frozen at −20° C for subsequent processing. The other part of the blood sample was used for Ficoll-Hypaque density gradient separation (Pharmacie LKV, Biotechnology AB, Vessala, Sweden) of cell series. The mononuclear blood cells thus obtained were adjusted to 5 × 106 cells/ml in phosphate-buffered saline solution and incubated for 2 hours in phytohemagglutinin (10 μL) and after washing were incubated for 24 hours in the presence of a constant concentration of *C. albicans* (4 × 108 colony-forming units/ml) obtained from a single colony. The concentration of IL-2 was measured in supernatants from lymphocyte cultures with *C. albicans* in both denture stomatitis and control groups.

IL-2 concentrations were measured in culture supernatant and serum with a sandwich-type immunoassay in 96-well microtiter plates that contained the anti–IL-2 monoclonal antibodies (Quantitative Human IL-2 Immunoassay, R&D Systems, Minneapolis, Minn.).

The results were analyzed with the help of the SSSP/PC+ statistical program.[18] The Mann-Whitney *U* test was used to compare the mean values and chi square analysis was used to study the association between qualitative variables. The associations were considered significant when $p < 0.05$.

RESULTS

In the study of local and general factors that might predispose the patient toward denture stomatitis, no significant differences were found between the stomatitis and control groups. Four patients had well-controlled diabetes. In the stomatitis group there were 20 nonsmokers and 9 patients who consumed more than 10 cigarettes per day. There were 11 nonsmokers, one light smoker (fewer than 10 cigarettes per day), and three moderate or heavy smokers (more than 10 cigarettes per day) in the control group. When dryness of mouth and denture age were studied, 22 patients had dryness of mouth and 7 patients had salivary flow within normal limits (nonstimulated salivary flow 0.25 to 0.35 ml/min). In the control group 8 patients had some degree of mouth dryness, and 7 patients had normal salivary flow. Eight patients in the stomatitis group and 11 control group patients had worn dentures for less than 5 years; 21 stomatitis patients and 4 control patients had worn dentures for longer. There were no significant differences between the stomatitis patient and control groups.

In the study of factors that might have affected IL-2 production, two stomatitis patients and two control patients exhibited antecedents of cellular hypersensitivity reaction to metals or atopic allergy to olive pollen. None of them were among the participants who had high serum or supernatant concentrations of IL-2.

Table I presents the results of *C. albicans* culture in Sabouraud medium and the concentrations of IL-2 in culture supernatant and serum. In the stomatitis group the pala-

Table I. Presence of *C. albicans* in palatal swab culture and concentration of IL-2 in serum and mononuclear blood cell culture supernatant

Stomatitis patient	*C. albicans* isolation	IL-2 serum levels (pg/ml)	IL-2 supernatant levels (pg/ml)	Control patient	*C. albicans* isolation	IL-2 serum levels (pg/ml)	IL-2 supernatant levels (pg/ml)
1	+	54	88	1	–	ND*	ND
2	+	ND	94	2	–	ND	ND
3	–	ND	ND	3	–	ND	ND
4	+	40	ND	4	+	ND	40
5	–	ND	ND	5	–	43	ND
6	+	ND	ND	6	+	90	42
7	+	95	40	7	+	150	75
8	–	45	ND	8	–	ND	ND
9	+	53	1000	9	–	ND	ND
10	+	40	2000	10	–	41	ND
11	–	75	ND	11	+	100	140
12	+	43	50	12	–	ND	ND
13	+	ND	990	13	–	ND	ND
14	+	75	200	14	–	ND	ND
15	–	42	ND	15	–	ND	40
16	–	40	ND				
17	+	200	40				
18	–	45	45				
19	+	ND	62				
20	–	ND	ND				
21	+	99	1000				
22	+	100	110				
23	–	ND	ND				
24	–	40	45				
25	+	90	95				
26	+	45	ND				
27	+	ND	ND				
28	+	100	200				
29	+	100	100				

ND, Not detected (<40 pg/ml).

tal swab from 19 patients grew *C. albicans*; there were four positive cultures in the control group. Statistical analysis revealed a significant relationship between denture stomatitis and *Candida infection* ($p < 0.014$) compared with the control group. In the stomatitis group serum concentrations ranged from 40 to 200 pg/ml (mean 71.1 pg/ml, SD 39.1 pg/ml). In controls IL-2 concentrations ranged from 41 to 150 pg/ml (mean 84.8 pg/ml, SD 45.2 pg/ml).

The concentrations in culture supernatants ranged from 40 to 2000 pg/ml in the stomatitis group and 40 to 140 pg/ml in the control group. The mean value in stomatitis group was 362.3 ± 552.8 pg/ml, and the corresponding value in control patients was 67.40 ± 43.2 pg/ml. A statistically significant difference between the two groups was found ($p < 0.05$). Fig. 1 illustrates the distribution of both groups according to IL-2 concentrations in supernatant from stimulated blood cell cultures. Detectable levels of cytokine were significantly more frequent in the stomatitis group ($p < 0.05$).

There was no significant relationship between the presence of *C. albicans* in the palatal swab and the serum IL-2 concentration in stomatitis or control patients. Fig. 2 illustrates the percentage of participants with normal or high IL-2 concentrations plotted against the presence of *C. albicans* in the palatal swab culture. However, supernatant concentrations of IL-2 were significantly related to the presence of *C. albicans* in the mouth, both in stomatitis ($p < 0.002$) and control ($p < 0.001$) groups (Fig. 3).

No significant relationship was found between subjectively painful symptoms of denture stomatitis and IL-2 concentrations in stimulated cultures of lymphocytes.

Patients with elevated serum titers of IL-2 might also have been expected to have elevated or at least detectable concentrations of the cytokine in culture supernatants. There was no apparent relationship between the two measures in 13 subjects. However, detectable IL-2 concentrations could be measured in both media in 14 of 29 patients.

When denture age was examined in relation to *C. albicans* infection, the relationship between these two parameters differed significantly between stomatitis and control groups (Fig. 4). In stomatitis patients positive palatal swab

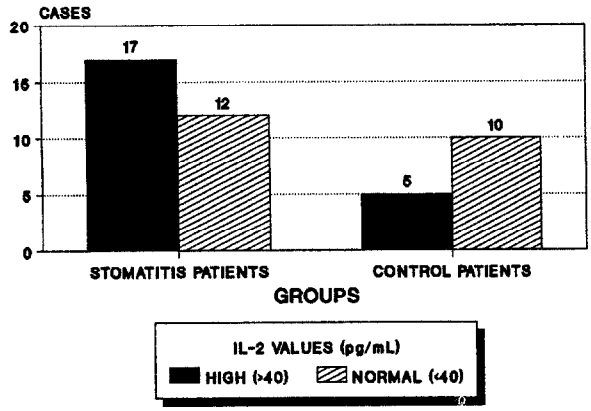

Fig. 1. Number of stomatitis and control patients with detectable (>40 pg/ml) or undetectable concentrations (<40 pg/ml) of IL-2 in supernatant of lymphocyte cultures in presence of *C. albicans*.

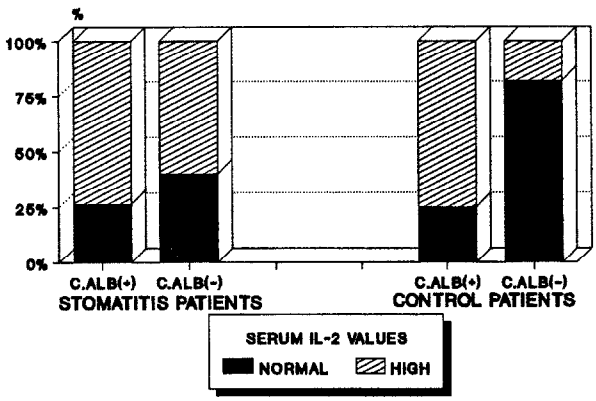

Fig. 2. Distribution of stomatitis and control patients according to serum concentration of IL-2 and results of palatal swab cultures to detect *C. albicans*.

cultures were more common in the group that had worn dentures longer than 5 years.

DISCUSSION

Although *C. albicans* has been traditionally blamed for denture stomatitis,[19] most research based this conclusion on the temporal coincidence of the two entities.[20, 21] However, coexistence does not necessarily indicate a cause-and-effect relationship between the two phenomena. Although it does not settle the question, this study provides new information on the mechanisms involved in the pathogenicity of *C. albicans*.[10] Several studies have related different components of the *C. albicans* cell wall to the modulatory effects on the immune system. In particular, cell wall polysaccharides are thought to cause T-lymphocyte immunosuppression and to enhance B-cell[22] and natural killer cell activation.[23] Domer et al.[24] found that mannose produced by these fungi comprised a heterogeneous mixture of components able to increase or inhibit the immune response. Although the mechanism of action of cell wall polysaccharides is poorly understood, stimulation of

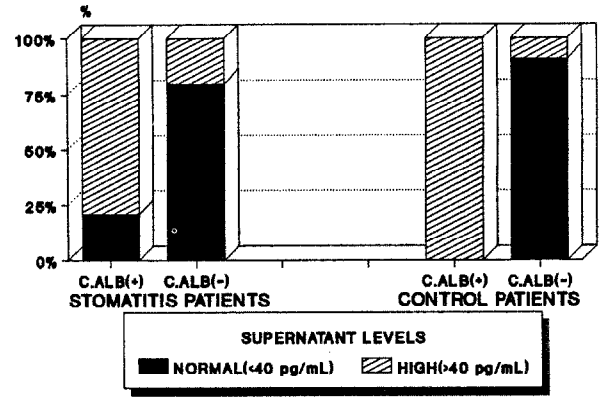

Fig. 3. Distribution of stomatitis and control patients according to concentration of IL-2 in culture supernatant and results of palatal swab cultures to detect *C. albicans*.

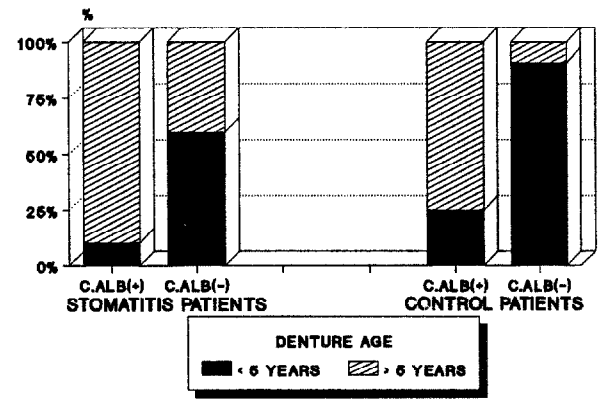

Fig. 4. Percentage of subjects in which *C. albicans* was isolated according to denture age.

the immune response probably does not reflect a direct mitogenic action on lymphocytes. Rather, it represents a mechanism that favors the production of factors, such as IL-2, that have this ability.[24] Our results support this hypothesis because IL-2 production was found to be greater in stomatitis and control groups whose palatal swab cultures were positive for *C. albicans* (Fig. 3).

Another finding in this study that supports the hypothesis of Domer et al.[24] was the significant difference between patients with denture stomatitis lesions and control patients without stomatitis in IL-2 production by cells grown in the presence of *C. albicans*. This result may be related to the importance of this cytokine as a mediator of inflammation. After antigen presentation in thymus-dependent antigenic stimuli, IL-2 secretion conditions, to a large extent, the response of macrophages and cytotoxic T lymphocytes. The IL-2 secretion conditions also directly affect lymphocyte proliferation.[25] Thus the immune response may be more intense for patients with IL-2 production that was markedly stimulated by cultured cells.[26]

Cantorna and Balish[5] demonstrated that CD4-positive T lymphocytes were involved in resistance to mucosal infection by *Candida* and that IL-2 and interferon gamma also

played a role in resistance. The role of IL-2 in the appearance of mucosal lesions caused by *Candida* remains obscure. Wang et al.[25] and Blanchard et al.[27] suggested a direct effect of colony-stimulating factor in increased resistance to infection and claimed that the role of IL-2 was secondary to the effect of this factor.

Analyses of IL-2 production after antigenic stimulation should involve patient groups with similar blood cell populations. If patients in whom cellularity and the proportions of cell populations differ markedly are compared, it is difficult to tell whether differences in stimulated IL-2 production are due to differences in the patient's capacity to react to stimulation or to different numbers of IL-2–producing cells.

When the association between in vitro production of IL-2 after *Candida albicans* stimulation and serum concentrations of cytokine was examined, 17 of 22 stomatitis and control patients who had detectable concentrations of IL-2 in the culture supernatant after stimulation also had detectable cytokine levels in serum. A significant relationship was found between supernatant and serum IL-2 levels. The high serum values may have reflected a transient increase in the intensity of the immune response as a result of systemic antigenic stimulation by *C. albicans*.

The significant association between the presence of *C. albicans* as detected in palatal swab or blood culture and increased IL-2 production in the supernatant of lymphocyte cultures strongly suggests that the immune systems of these patients conserve active memory of *Candida* stimulation, regardless of whether they have denture stomatitis. There was no in vitro production of IL-2 in a larger percentage of stomatitis and control patients with negative palatal swab culture results. Because of the greater age of the population in this study, it is possible that some patients had a previous *Candida* infection. If this were so, it would suggest that the immune system's memory of these antigens is limited in time and that the duration of prestimulation by these antigens is brief.

In a study of blood cell stimulation with *Candida* spp. Gauchat et al.[23] found increased interferon gamma and IL-2 production, although the increases were not so marked as they were in this study. Wang et al.[25] and Blanchard et al.[27] reported increased production of colony-stimulating factor and interleukin-l by cells grown in vitro with *C. albicans*; this response would also bring about an increase in IL-2 production. No significant relationship was found between etiopathogenic factors of candidiasis and IL-2 production. This result suggests that such factors do not condition IL-2 production and that the values found did not reflect the effect of etiopathogenic features.

In an earlier study of the effects of denture age, an increase in cultures positive for *Candida* was found in patients who had worn dentures for more than 5 years.[12] In this study *C. albicans* was found in 52% of the patients reported; of this proportion 87% had worn dentures for more than 5 years. There was no significant difference in stimulation of IL-2 production in vitro between populations with *Candida* infection who had used dentures for fewer than or more than 5 years.

CLINICAL SIGNIFICANCE

Studies reported here show the importance of IL-2 production induced in T lymphocytes previously cultured in presence of *C. albicans* in denture stomatitis lesions. These results represent a first step in the understanding of mechanisms involved in the etiology of this disorder. In the future dentists will have the opportunity to use immunomodulatory drugs for the rational treatment of denture stomatitis.

CONCLUSIONS

In this study no significant relationship was found between serum IL-2 levels and denture stomatitis. Nevertheless, there was a significant relation between in vitro production of IL-2 by T lymphocytes previously stimulated with *C. albicans* and denture stomatitis lesions. These results suggest that *Candida* infection plays a important role in the immunomodulation of this cytokine and in the appearance of denture stomatitis lesions.

We thank Ms. Gertrudis Gomez-Villaescusa for her technical assistance and Ms. Karen Shashok for translating the original manuscript into English.

REFERENCES

1. Dorey JL, Blasberg B, MacEntee MI, Conklin RJ. Oral mucosal disorders in denture wearers. J PROSTHET DENT 1985;53:210-3.
2. Fouche MH, Slabbert JC, Coogan MM. Microorganisms isolated from patients with denture stomatitis. J Dent Assoc South Africa 1986;41:313-6.
3. Koopmans AS, Kippuw N, de Graaff J. Bacterial involvement in denture-induced stomatitis. J Dent Res 1988;67:1246-50.
4. Theilade E, Budtz-Jorgensen E. Predominant cultivable microflora of plaque on removable dentures in patients with denture-induced stomatitis. Oral Microbiol Immunol 1988;3:8-13.
5. Cantorna MT, Balish E. Role of CD4+ lymphocytes in resistance to mucosal candidiasis. Infect Immun 1991;59:2447-55.
6. Ceballos A, Urquia M. Delayed cellular hypersensitivity against *Candida albicans* in patients wearing dentures [in Spanish]. Rev Act Odontoestomatol Espan 1990;390:47-50.
7. Csato M, Kenderessy AS, Judak R, Dobozy A. Inflammatory mediators are involved in the *Candida albicans* killing activity of human epidermal cells. Arch Dermatol Res 1990;282:348-50.
8. Torosantucci A, Palma C, Boccanera M, Ausiello CM, Spagnoli GC, Cassone A. Lymphoproliferative and cytotoxic responses of human peripheral blood mononuclear cells to mannoprotein constituents of *Candida albicans*. J Gen Microbiol 1990;136:2155-63.
9. Wei S, Serbousek D, McMillen S, Blanchard DK, Djeu JY. Suppression of human monocyte function against *Candida albicans* by autologous IL-2–induced lymphokine-activated killer cells. J Immunol 1991; 146:337-42.
10. Scaringi L, Rosati E, Cornacchione P, Rossi R, Marconi P. In vivo modulation of lymphokine-activated killer cell activity by cell wall components of *Candida albicans*. Cell Immunol 1992;139:438-54.
11. Podzorski RP, Herron MJ, Fast DJ, Nelson RD. Pathogenesis of candidiasis: immunosuppression by cell wall mannan catabolitis. Arch Surg 1989;124:1290-4.
12. Ceballos A, Gonzalez-Moles M, Urquia M. Denture stomatitis: its relation to *Candida albicans* [in Spanish]. Av Odontoestom 1990;6:151-4.
13. Fischer A, Pichay L, Audinot M, Griscelli O. Defective handling of

mannan by monocytes in patients with chronic mucocutaneous candidiasis resulting in a specific cellular unresponsiveness. Clin Exp Immunol 1987;47:653-60.
14. Ausiello CM, Urbani F, Gessani S, Spagnoli GC, Gomez MJ, Cassone A. Cytokine gene expression in human peripheral blood mononuclear cells stimulated by mannoprotein constituents from *Candida albicans*. Infect Immun 1993;61:4105-11.
15. Budtz-Jorgensen E. The significance of *Candida albicans* in denture stomatitis. Scand J Dent Res 1974;82:151-90.
16. Damle NK, Childs AL, Doyle LV. Immunoregulatory T lymphocytes in man: soluble, antigen-specific suppressor-inducer T lymphocytes are derived from the CD4+ CD45R-p80 J Prosthet Dent + subpopulations. J Immunol 1987;139:1501-8.
17. Treseler CB, Maziarz RT, Levitz SM. Biological activity of interleukin-2 bound to *Candida albicans*. Infect Immun 1992;60:183-8.
18. Norusis MJ. SPSS/PC+ statistics 4.0. Chicago: SPSS, 1991.
19. Cumming CG, Wight C, Blackwell CL, Wray D. Denture stomatitis in the elderly. Oral Microbiol Immunol 1990;5:82-5.
20. Budtz-Jorgensen E. *Candida* associated denture stomatitis and angular cheilitis. In: Samaranayake LP, MacFarlane TW. Oral candidosis. London: Wright, 1990:156-73.
21. Cardash HS, Helft M, Shani A, Marshak B. Prevalence of *Candida albicans* in denture stomatitis in an Israeli geriatric hospital. Gerodontology 1989;4:101-7.
22. Mangeney M, Fisher A, Le Deist F, Latge JP, Durandy A. Direct activation of human B lymphocytes by *Candida albicans*–derived mannan antigen. Cell Immunol 1989;122:329-37.
23. Gauchat JF, Gauchat D, De Weck AL, Stadler BM. Cytokine mRNA levels in antigen-stimulated peripheral blood mononuclear cells. Eur J Immunol 1989;19:1079-85.
24. Domer J, Elkins K, Ennist D, Baker P. Modulation of immune responses by surface polysaccharides of *Candida albicans*. Rev Infect Dis 1988;10(suppl 2):S419-22.
25. Wang M, Friedman H, Djeu JY. Enhancement of human monocyte function against *Candida albicans* by the colony-stimulating factors (CSF): IL-3, granulocyte-macrophage-CSF and macrophage CSF. J Immunol 1989;143:671-7.
26. Roitt I, Brostoff J, Male DK. Immunology. 2nd ed. London: Gower Medical, 1989.
27. Blanchard KD, Michelini-Norris MB, Djeu JY. Production of granulocyte-macrophage colony-stimulating factor by large granular lymphocytes stimulated with *Candida albicans*: role in activation of human neutrophil function. Blood 1991;10:2259-65.

Reprint requests to:
DR. MIGUEL URQUIA GARCIA
MARQUES DE LA ENSENADA N° 4 ESC. B 5° B
E-18004, GRANADA
SPAIN

CONTRIBUTING AUTHOR

Cristina Citoler, BDS, Scholar, Department of Oral Medicine, School of Dentistry, University of Granada.