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Immunological Activities of a *Candida albicans* Protein Which Plays an Important Role in the Survival of the Microorganism in the Host

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A protein with an isoelectric point of 4.3 and a relative molecular mass of 43 kDa (p43) was purified from the supernatants of the cultures of pathogenic *Candida albicans* but could not be detected in the supernatants of cultures of this fungus with pathogenicity previously attenuated after being repeatedly subcultured in vitro. Treatment of BALB/c and C57BL/6 mice with p43 resulted in (i) marked increases in the numbers of splenic immunoglobulin-secreting plaque-forming cells (PFC) with peak responses of immunoglobulin M (IgM) PFC preceding those of IgG PFC, with an isotype restriction pattern of IgG2a>IgG2b>IgG3>IgG1>IgM, and (ii) specific immunosuppression of the murine primary immune response against sheep erythrocytes. Immunosuppressive and B-cell mitogenic properties of p43 were quantitatively associated and inversely correlated with susceptibility to *C. albicans* infection. C57BL/6 mice treated with p43 2 days before inoculation with *C. albicans* were considerably more susceptible to the fungal infection than untreated mice. The immunobiological and chemical properties of p43 are compared with previously described immunosuppressive and B-cell mitogenic proteins produced by bacteria and viruses, and strategies for immunointervention are discussed.

It is well-known that the commensal fungus *Candida albicans* is an opportunistic pathogen in the immunodeficient host. It has also been observed that sera of patients with chronic mucocutaneous candidiasis were able to suppress immune responses specific for *C. albicans* (4, 16). Furthermore, *C. albicans* or selected components thereof, such as mannan, other polysaccharides, cell wall glycoproteins, and fungal toxins or metabolites have been shown to induce immunosuppression (10, 21, 30, 32). On the other hand, increased resistance to challenge with *C. albicans* was achieved by treatment with anti-μ chain antibodies (23) and with immunosuppressive drugs such as cyclophosphamide (6, 9) or nitrogen mustard (20). Infection with *C. albicans* has also been shown to enhance the proliferative ability of lymphocytes from immunologically immature germ-free rats in response to mitogens (33) and to potentiate antibody responses in mice to antigens unrelated to the fungus as well (12). Finally, cell wall-derived mannoproteins or glucan fractions appear to activate natural killer and macrophage cell populations (36).

Similar contrasting observations have also been reported in other microbial infections. Thus, mice that are genetically B-cell deficient (Xid mice) or depleted of B cells by anti-μ treatment have increased resistance to *Trypanosoma cruzi* (28), *Leishmania donovani* (34), and *Plasmodium chabaudi adami* (19). Moreover, it is well-known that B-cell mitogenicity has been associated with numerous microorganisms and that a quantitative correlation appears to exist between immunosuppression and B-lymphocyte mitogenicity (1–3, 14). Some such activities have been ascribed to lipopolysaccharide (38, 40) and to proteins produced by *Streptococcus intermedius* (1, 2), *Streptococcus mutans* (14), and the African swine fever virus (3, 31).

Here we report a B-cell mitogenic and immunosuppressive protein produced by *C. albicans* which appears to play an important role in the protection of the microorganism from the defense mechanisms of the host.

MATERIALS AND METHODS

Mice. Male BALB/c and C57BL/6 mice, 8 to 10 weeks old, were bred at the Gulbenkian Institute, Oeiras, Portugal.

C. albicans. *C. albicans*, identified according to the taxonomic criteria of Lodder (24), was isolated from a patient afflicted with generalized mucocutaneous candidiasis and maintained by passages every 3 weeks in Sabouraud dextrose agar medium (Difco Laboratories, Detroit, Mich.). The pathogenicity of the fungus was maintained by inoculations of *C. albicans* into C57BL/6 mice every 3 months and subsequent recovery of the fungus from the infected kidneys. An attenuated form of the same isolate of *C. albicans* was obtained after 60 passages in Sabouraud dextrose agar medium over 8 months.

Preparation of the product(s) secreted by *C. albicans*. *C. albicans* was cultured in Sabouraud dextrose agar medium for 24 h at 28°C and then transferred to a medium containing 0.3% (wt/vol) yeast extract (Difco) and 0.2% (wt/vol) glucose (Winge medium [WM]) for another 60 h. The last incubation was performed at 37°C with constant agitation at an initial concentration of 10^8 particles per liter of medium. The cultures were then centrifuged at 2500 × *g* for 30 min, and the supernatants were filtered through 0.45-μm-pore-size filters (Millipore) before being concentrated by vacuum dialysis through a Visking 100/8FT dialysis membrane with a 12,000 Da cutoff.

Fractionation by ion-exchange chromatography. Culture supernatants from both the pathogenic and attenuated *C. albicans* cultures were fractionated by ion-exchange chromatography in DEAE-Sephadex A 50 (Pharmacia Fine Chemicals, Inc., Piscataway, N.J.) by using a discontinuous molarity gradient of 0.05, 0.5, 1, and 2 M NaCl with neutral

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pH by a method previously described in detail (3, 31). The separated extracellular products of *C. albicans* (EP-Ca) were designated F0.05M to F2M EP-Ca path or inact, representing products from pathogenic or attenuated cultures, respectively. WM was fractionated by the same procedure, and the 0.05 and 0.5 M fractions were pooled together and designated F0.05+F0.5M WM for use as controls. All fractions were dialyzed against phosphate-buffered saline (PBS) and reconstituted by vacuum dialysis as culture supernatants to a volume equivalent to 400 μg of protein per ml. This was approximately the same proteinaceous concentration of the original EP-Ca and WM. Protein concentration was determined by the method of Lowry et al. (26).

Removal of mannoside constituents from the biologically active fractions. Because the immunosuppressive activity was detected in F0.05M and F0.5M EP-Ca path, 1 mg of F0.05+0.5M path protein was passed through a 5-ml concanavalin A-Sepharose column (Sigma Chemical Co, St. Louis, Mo.) previously equilibrated with 0.05 M Tris-HCl buffer (pH 7.5) supplemented with 10 mM $MgCl_2$. The sugar-depleted subfraction was designated F'p EP-Ca path, as it was made up of proteinaceous material obtained by washing the column with the same Tris-HCl buffer. The sugar-enriched subfraction, F's EP-Ca path, was eluted with 15 ml of 0.5 M methyl-α-D-mannoside over 2 h. Both subfractions were dialyzed against PBS and concentrated by vacuum dialysis. F'p EP-Ca path was adjusted to 200 μg of protein per ml. F's EP-Ca path was concentrated to the same volume of F'p EP-Ca path and contained 340 μg of protein (glycoprotein) per ml.

Purification of the immunosuppressive factor. The immunosuppressive activity was detected exclusively in F'p EP-Ca path, and the biologically active protein was purified from this subfraction by polyacrylamide gel electrophoresis (PAGE) (13, 29) performed in 7.5% polyacrylamide gels with a prerun of 2 h to remove excess of *N,N,N',N'*-tetramethylenediamine (TEMED). After PAGE, the gels were cut longitudinally into two halves, one of which was stained with silver nitrate, methylene blue, or periodic acid-Schiff. Constituents in the unstained portion of the gel which corresponded to the silver nitrate-stained bands in the other half were eluted by electrophoresis (39). Each subfraction obtained by this method was dialyzed against PBS, concentrated by vacuum dialysis, and diluted to a proteinaceous concentration of 100 μg/ml.

Analysis of the purified biologically active protein. Analytical isoelectric focusing of the purified biologically active protein and of a mixture of proteins of known isoelectric point was performed with ampholytes of pH 3 to 9 in strips (Phastgel IEF 3–9; Pharmacia) by using computerized systems for the separation and staining (Phast system separation control and development units; Pharmacia). After a prerun of 10 min, 1 μl of each sample was applied, and analytical isoelectric focusing was performed over 30 min. These gel strips were also stained with silver nitrate, methylene blue, or periodic acid-Schiff. The relative mass of this protein was determined by sodium dodecyl sulfate (SDS; Sigma)-PAGE (41) with proteins of known relative masses as references. SDS-PAGE preparations were stained with silver nitrate.

Enzymatic treatment. Protease type XVII from *Staphylococcus aureus* V8 (Sigma) and endoglycosidase H (Boehringer Mannheim GmbH, Mannheim, Germany) were used for enzymatic treatment of samples. Samples were treated for 12 h at 37°C in PBS at pH 7.2 with enzymes at concentrations equivalent to 1/50 of the amount of the protein in the substrate.

Immunization, infection, and treatment protocols. BALB/C and C57BL/6 mice were immunized by injecting 5×10^7 sheep erythrocytes (SRBC) intraperitoneally (i.p.), and the direct and indirect splenic plaque-forming cell (PFC) responses were assayed before and after immunization. Mice were inoculated with pathogenic or nonpathogenic *C. albicans* by i.p. injection of either 1×10^7 or 5×10^7 microorganisms previously cultured in Sabouraud dextrose agar for 24 h at 28°C. Both kidneys were removed aseptically at selected times after inoculation, homogeneized in 5 ml of PBS, and diluted serially five times. CFU of *C. albicans* were counted 48 h after duplicated cultures of each serial dilution in Mycobiotic agar (Difco). Kidneys were also fixed in Bouin for 24 h and stained either by hematoxylin-eosin or by the method of Gridley (17). Mice were injected i.p. with 200 μg (each) of F0.05M, F0.5M, F1M, and F2M EP-Ca path or F0.05M, F0.5M, F1M, and F2M inact protein or with proportionally lower amounts of the subfractions, until 50 μg of the purified biologically active molecule was reached, 2 days before immunization with SRBC or fungal inoculation with *C. albicans*. As controls, mice were treated i.p. with a single injection of 200 μg of F0.05+F0.5M WM protein. In the case of enzyme treatment, control mice were also injected i.p. with the enzyme used (proteinase type VII or endoglycosidase H) at a concentration of 1/50 of the treated sample.

PFC assays. Production of specific anti-SRBC immunoglobulin M (IgM) and IgG antibodies was evaluated by direct and indirect hemolytic PFC assays, respectively (7). Nonspecific immunoglobulin production was assessed by the protein A PFC assay (18). Rabbit antisera against total immunoglobulin or specific for each isotype were used as developing antibodies and were previously characterized in detail (15).

RESULTS

Detection of immunosuppressive activities in fractions of culture supernatants derived from pathogenic and nonpathogenic *C. albicans*. The pathogenicity and attenuated character of the two *C. albicans* cultures were demonstrated by injecting 10^7 yeast cells i.p. into BALB/c mice and culturing their kidneys at selected intervals thereafter. As shown in Fig. 1, the attenuated culture did not become established in the kidneys over time, whereas the pathogenic culture did. When each of these cultures was employed to obtain and fractionate extracellular protein, none of the fractions from the attenuated culture was suppressive when compared with untreated mice or with mice treated with F0.05+0.5M WM as controls. The F0.05M and F0.5M fractions from the pathogenic culture markedly suppressed the primary immune response against SRBC in C57BL/6 mice (Table 1).

Characterization of the immunosuppressive protein. As shown in Table 1, after subfractionation of the mixture of F0.05+F0.5M EP-Ca path into sugar-enriched F's EP-Ca path and sugar-depleted F'p EP-Ca path subfractions, the immunosuppressive effect was detected only in F'p EP-Ca path. The analysis of F'p EP-Ca path by classical PAGE revealed two silver nitrate-stainable bands (Fig. 2). The immunosuppressive activity was detected in the material electroeluted from the more-anodal band (Table 1). This material was designated p43 for several reasons; though the immunoregulatory activity was retained after heating at 60°C for 60 min (Table 1), this band was degradated by protease

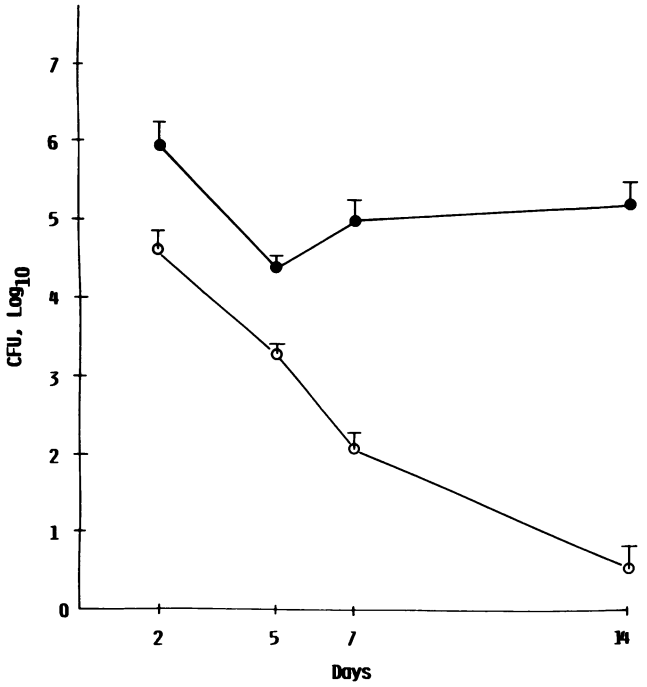

FIG. 1. Growth of pathogenic (●) and attenuated (○) *C. albicans* in the kidneys of BALB/c mice, expressed as CFU, at selected intervals after i.p. injection of 10^7 *Candida* particles. This is a representative experiment repeated three times with different inoculum sizes. In each experiment, the results are means of four mice and one standard deviation.

TABLE 1. Splenic anti-SRBC IgM-secreting PFC in mice[a]

Treatment or fraction	Protein (μg)	Log_{10} IgM-secreting PFC per spleen[b]
Untreated	0	6.00 ± 0.07
F0.05+F0.5M WM	200	5.95 ± 0.05
Protease type XVII B	1	5.88 ± 0.10
Endoglycosidase H	1	6.09 ± 0.20
F0.05M EP-Ca inact	200	5.97 ± 0.03
F0.5M EP-Ca inact	200	5.99 ± 0.07
F 1M EP-Ca inact	200	5.88 ± 0.08
F 2M EP-Ca inact	200	5.82 ± 0.15
F0.05M EP-Ca path	200	3.32 ± 0.58[c]
F0.5M EP-Ca path	200	3.72 ± 0.63[c]
F 1M EP-Ca path	200	5.95 ± 0.13
F 2M EP-Ca path	200	5.89 ± 0.14
F'p EP-Ca path	100	3.69 ± 0.27[c]
F's EP-Ca path	170	6.00 ± 0.09
p43	50	3.19 ± 0.86[c]
p43 heated for 60 min at 70°C	50	3.24 ± 0.57[c]
p43 treated with protease type XVII B	50	5.61 ± 0.21
p43 treated with endoglycosidase H	50	3.32 ± 0.66[c]

[a] Data are from 5 days after SRBC immunization of untreated C57BL/6 mice or mice treated 2 days before immunization with a single i.p. injection of the designated fractions.

[b] Results are from a representative experiment repeated three times. Results are means ± one standard deviation of three mice.

[c] Statistically significant; $P < 0.001$ compared with untreated or F0.05+0.5M WM controls.

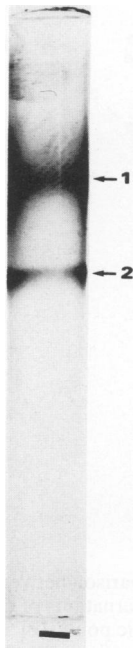

FIG. 2. Pattern of PAGE analysis of F'p EP-Ca showing two silver nitrate-stainable bands (bands 1 and 2). The immunosuppressive activity was detected in the material corresponding to the more anodal (−) band, band 2.

type XVII B but not by endoglycosidase H (Fig. 3), and the biological activity of the material was abrogated by treatment with the proteolytic enzyme but was resistant to treatment with the glycolytic enzyme (Table 1). No significant differences were observed in the numbers of splenic PFC in untreated or treated mice with proteolytic or glycolytic enzymes (Table 1). The material reacted with silver nitrate but not with periodic acid-Schiff or methylene blue, emphasizing its proteinaceous nature. The protein had an isoelectric point of 4.25 (Fig. 4A), as determined by analyt-

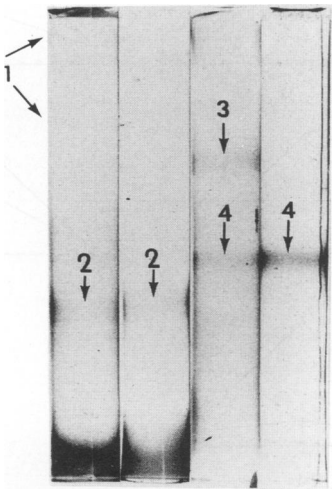

FIG. 3. Pattern of PAGE analysis of p43 after enzymatic treatment with protease B XVII (band 1) and after endoglycosidase (band 2). The bands observed after PAGE, corresponding to protease B XVII (band 3) and to endoglycosidase (band 4), are also shown after incubation with p43 or alone.

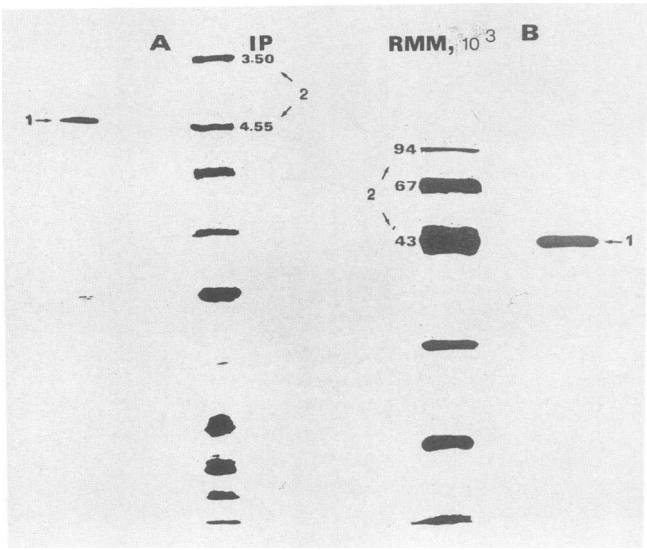

FIG. 4. Analytical comparison between the most purified protein (p43) obtained from the supernatants of *C. albicans* and a mixture of proteins of known isoelectric point (IP) and relative molecular mass (RMM). (A) Isoelectric focusing of p43 (band 1) and of standard proteins (band 2) with IP is indicated. (B) SDS-PAGE of p43 (band 1) and of standard proteins with indicated RMM (band 2).

ical isoelectric focusing, and a relative molecular mass of 43,000 Da (43 Kda), as determined by SDS-PAGE (Fig. 4B).

Immunosuppressive effects of p43 on nonspecific immunoglobulin production in SRBC-immunized and unimmunized mice. BALB/c and C57BL/6 mice, one-half of which had been treated with p43 2 days prior to immunization, were immunized with SRBC, and the production of nonspecific and SRBC-specific immunoglobulin was determined. As shown in Fig. 5, a marked increase in the numbers of splenic nonspecific IgG and IgM-secreting PFC was observed by protein A PFC assay, accompanying specific immunosuppression observed on day 5 in SRBC-immunized mice treated with p43. The increase in the numbers of nonspecific immunoglobulin-secreting PFC reached the highest values 3 days after immunization and preceded the specific immunosuppression which was also preceded by a slight enhancement of the specific responses. In general, the higher the increase in the numbers of a given nonspecific immunoglobulin-secreting PFC, the stronger was the suppression of specific anti-SRBC immunoglobulin-secreting PFC of the corresponding isotype observed in both strains of mice. Interestingly, as also shown in Fig. 5, both the nonspecific and specific immunosuppressive PFC responses to p43 were more pronounced in the strain of mice (BALB/c) that is more sensitive to *C. albicans* infection, i.e., the numbers of fungal CFU were 3 log units higher in the kidneys of BALB/c than of C57BL/6 mice 7 days after infection with 5×10^7 fungal particles.

As shown in Fig. 6 and Table 2, dramatic increases in the numbers of nonspecific protein A, splenic immunoglobulin-secreting PFC were also observed in both unprimed C57BL/6 and BALB/c mice after p43 treatment. As in the case of primed mice, these responses were, with the exception of IgG2b-secreting PFC, even higher (up to 320 times higher than controls in the case of IgG2a-secreting PFC) and more prolonged in time in BALB/c than in C57BL/6 mice. In both strains of mice, the nonspecific peak responses of IgM preceded those of IgG isotypes, and the isotype restriction pattern, taken on peak responses, was IgG2a>IgG2b>

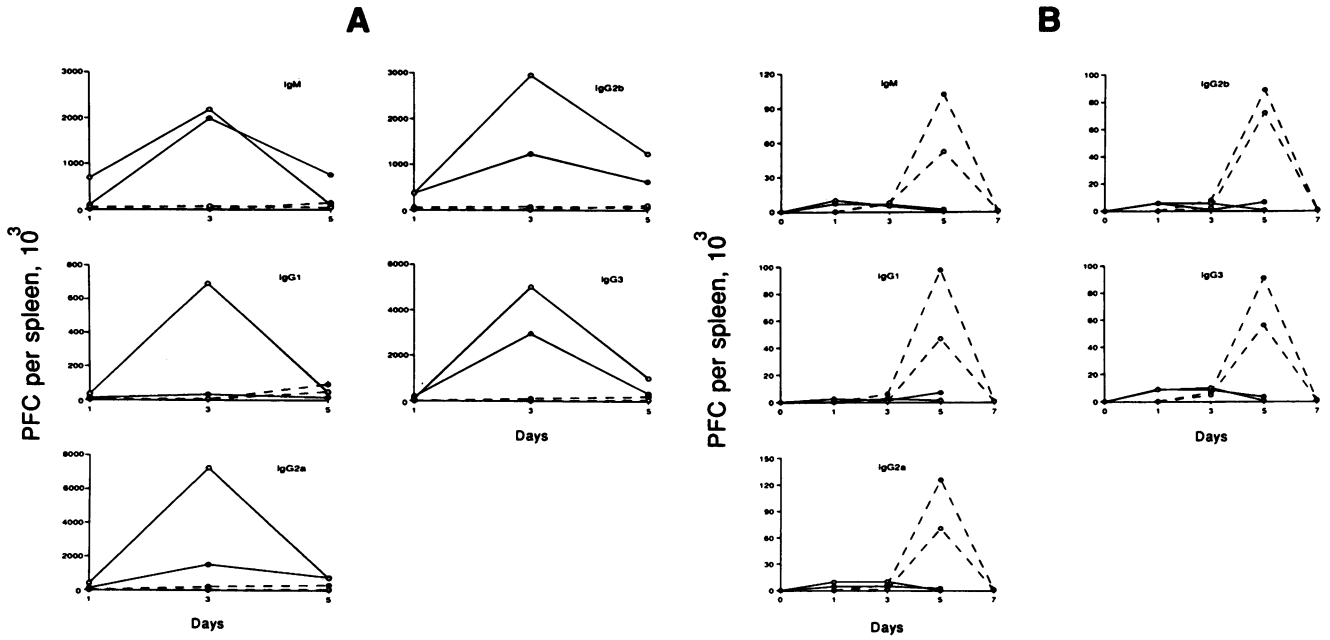

FIG. 5. Numbers per spleen of nonspecific (A) and specific (B) anti-SRBC immunoglobulin-secreting PFC of the indicated isotypes observed in SRBC-primed C57BL/6 (●) and BALB/c (○) mice either treated (——) or untreated (- - -) with p43 on the indicated days after immunization. Here and in Fig. 6, results are means of three mice and represent, as in Fig. 1, one typical experiment performed three times. Also, here and in Fig. 6, as the standard deviations never exceeded 20% of the mean of the PFC assays and in 90% of the cases were less than 10%, they were omitted for simplicity.

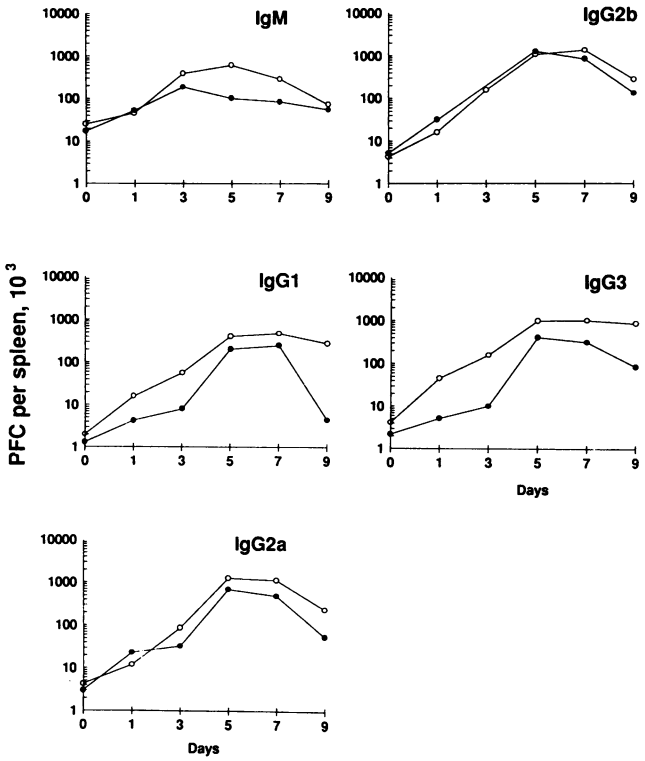

FIG. 6. Numbers per spleen of nonspecific immunoglobulin-secreting PFC of the indicated isotypes observed in SRBC-unprimed C57BL/6 (●) and BALB/c (○) mice, on the indicated days after p43 treatment. See legend to Fig. 5.

IgG3>IgG1>IgM (Fig. 6 and Table 2). No significant increases in IgA-secreting PFC were observed in either primed or unprimed mice after treatment with p43 (data not shown).

Role of p43 in the protection of the microorganism in the host. C57BL/6 mice, relatively resistant to *C. albicans*, were considerably more susceptible to fungus infection when treated 2 days before fungal challenge with 50 μg of p43 protein. This increase in susceptibility was specific, since it did not occur when mice were treated with up to 200 μg of F0.05+F0.5M WM nonmitogenic protein or when they were left untreated. Susceptibility was demonstrated by a significant increase in the numbers of CFU found in the kidney 2,

TABLE 2. Peak responses of nonspecific immunoglobulin-secreting PFC of the different isotypes observed in nonimmunized p43-treated BALB/c and C57BL/6 mice

Mouse line and treatment	Log_{10} nonspecific PFC per spleen[a]				
	IgM	IgG1	IgG2a	IgG2b	IgG3
BALB/c					
p43 treated	5.98	5.63	6.32	6.30	5.99
Control	4.58	3.25	3.82	3.89	3.60
C57BL/6					
p43 treated	5.32	5.47	5.75	6.26	5.60
Control	4.28	3.23	3.38	3.91	3.35

[a] Results were taken from Fig. 6. The significant higher differences between BALB/c and C57BL/6 mice in the numbers of nonspecific PFC observed on the peak responses are underlined ($P < 0.001$). The significance was calculated by the Student *t* test.

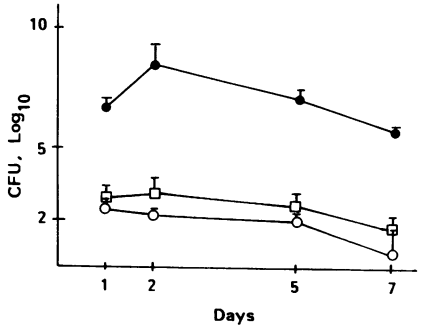

FIG. 7. Mean CFU of *C. albicans* observed, on the indicated days after i.p. infection with 5×10^7 particles of pathogenic *C. albicans*, in the kidneys of C57BL/6 mice either untreated (□) or treated with F0.05+0.5M WM protein (○) or with p43 (●) 2 days before the inoculation of *C. albicans*. Results are means and one standard deviation for four mice.

5, and 7 days after the infection (Fig. 7). Furthermore, the histopathological study of the kidneys of p43-treated C57BL/6 mice performed 5 days after *C. albicans* infection revealed a characteristic pattern of acute candidiasis, viz., the presence of *Candida* pseudohyphae and blastoconidia and hyphae in the kidney medulla and cortex, as well as necrotic regions (Fig. 8). In contrast, the fungal elements were confined to the medulla in mice treated with F0.05+F0.5M WM protein (Fig. 8). Moreover, no necrotic regions or fungal infiltrations were observed in the cortex of these mice. In contrast to the mice treated with F0.05+F0.5M WM, marked infiltration of mononuclear cells in the cortex and medulla was observed in the hematoxylin-eosin-stained preparations from mice treated with p43 (data not shown).

DISCUSSION

The present results indicate that, in analogy to other microorganisms (2, 3, 14), *C. albicans* produces an immunosuppressive and B-cell mitogenic protein. These p43 effects cannot be interpreted as mere antigenic competition, as control mice were treated with even larger amounts of protein from tissue culture medium of *C. albicans*. Indeed, treatment with this amount of WM protein did not induce significant differences from the unmanipulated mice in the numbers of both SRBC-specific and nonspecific PFC. Moreover, it was previously observed that no such immunosuppressive effects were observed when mice were treated with larger amounts of protein with similar relative molecular masses (31). The immunosuppressive and B-cell mitogenic effects of p43 were quantitatively associated. Similar quantitative association has also been ascribed to other proteins produced by other microorganisms (3, 14). These findings are compatible with the possibility that B-cell polyclonal activation is the cause of the specific immunosuppression induced by p43 and the other described immunosuppressive and B-cell mitogenic proteins. In fact, it was previously demonstrated that B-cell activation was a crucial step in the process of immunosuppression induced by such proteins (2, 14, 31). Therefore, overstimulation of the immune system seems to be the mechanism by which immunosuppression is induced by p43 and by the other mitogenic proteins. In agreement with this assumption is the observation that the strong immunosuppression of the peak responses against SRBC (day 5) induced by p43 is preceded by an increase on

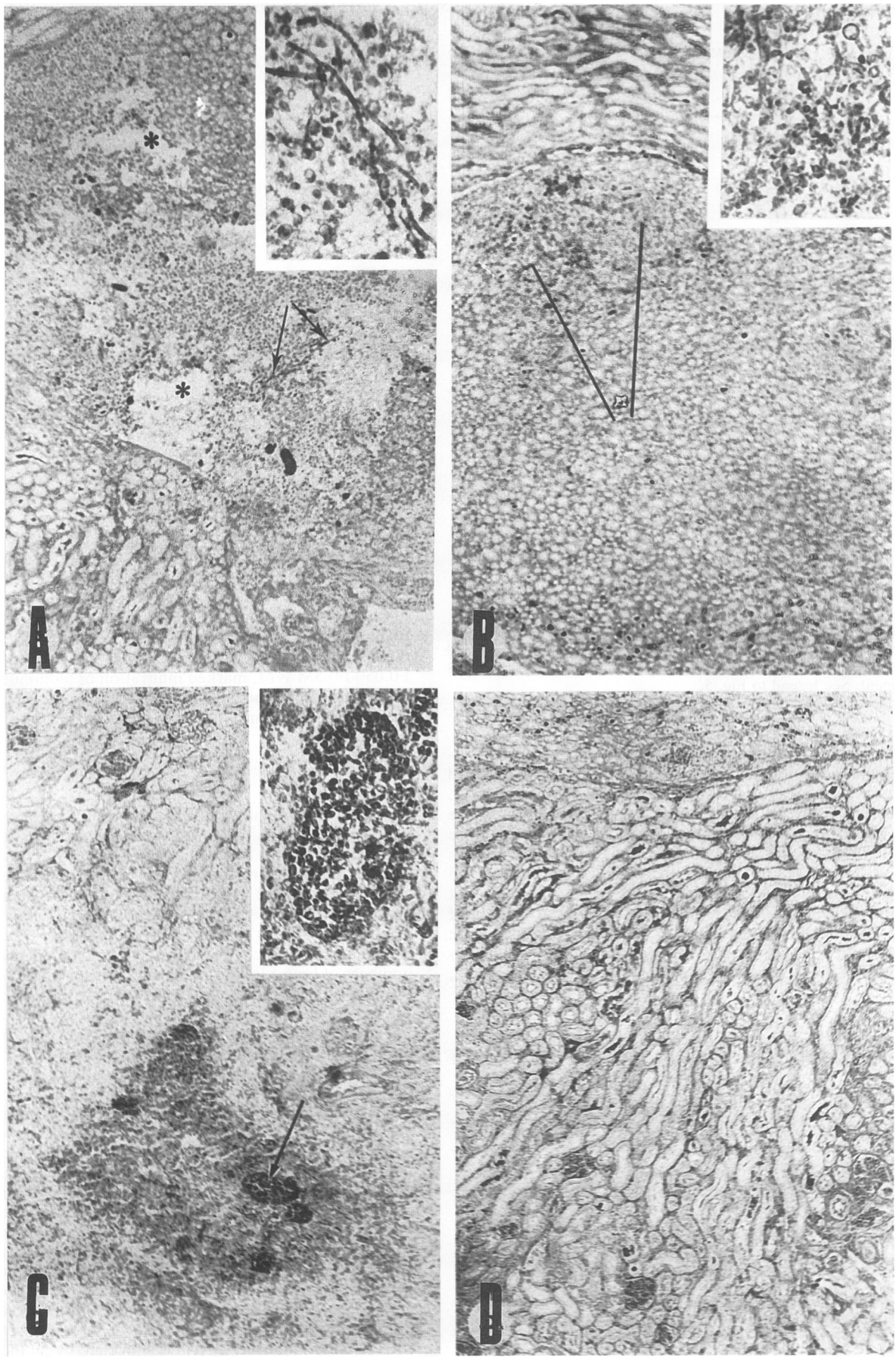
A
B
C
D

FIG. 8. Histopathological patterns of the kidney medulla of C57BL/6 mice treated 2 days before *C. albicans* infection with either p43 (A) or with F0.05+F0.5M WM protein (controls [B]). (C and D) Kidney cortex of the same infected mice treated (C) or untreated (D) with p43. The major histopathological preparations are amplified 450×, and the inserts were amplified 1,800×. The amplified inserts represent the tissue areas indicated by arrows in panels A and C and delimited by two convergent straight lines in panel B. The diffuse fungal infiltration areas consisting in blastoconidia and hyphae are shown in the insert of panel A. This diffuse fungal infiltration is accompanied by necrotic regions (*) (A), whereas it is delimited in the controls (B and respective insert). Extensive infiltration of *C. albicans* was observed in the kidney cortex of p43-treated mice (C and respective insert). In contrast, no fungal infiltration was observed in the kidney cortex of control mice (D).

day 1 in the numbers of splenic immunoglobulin-secreting PFC. The immunosuppressive effect of p43 plays an important role in the protection of the microorganism in the host. Indeed, p43 was undetectable in the supernatants of cultures of *C. albicans* with attenuated pathogenicity after being repeatedly subcultured. Moreover, both the immunosuppressive and B-cell mitogenic effects of p43 were more evident in BALB/c than in C57BL/6 mice, and the former strain of mice was more susceptible to our *C. albicans* inocula. Hector et al. (20) reported opposite results of mouse strain susceptibility to this fungus, but other reports (5, 9) are in agreement with our findings. Furthermore, susceptibility to the fungal infection of the strain found relatively resistant (C57BL/6 mice) was dramatically increased by treatment with p43 2 days before *C. albicans* infection. Indeed, a significant increase of CFU was observed in the kidneys of these mice accompanied by histopathological pattern of this organ characteristic of acute candidiasis which was undetectable in p43-untreated mice. Enhanced survival of *S. mutans* in the host was also achieved by an analogous immunosuppressive and B-cell mitogenic protein produced by that microorganism (35, 37). However, this protective effect is more clearly demonstrated in the *C. albicans* infection model because this fungus is distinctly more pathogenic in mice than *S. mutans* is.

Several observations are in agreement with the role of B-cell mitogenicity in the immunosuppression induced by microorganisms which subsequently increase their survival in the host. Indeed, impaired infections to protozoa, plasmodia, and helminths were seen in mice depleted of B cells by anti-μ chain or in animals genetically B-cell deficient, such as Xid mice (19, 28, 34). The predominant splenic nonspecific synthesis of IgG2a and IgG2b isotypes induced by p43 is also observed after murine treatment with the B-cell mitogenic proteins produced by streptococci (2, 14) and viruses (3). Moreover, a similar isotype restriction pattern is also observed after viral (11), protozoan (28), or helminth (25) infections. Interestingly, p43 is biochemically rather similar to the immunosuppressive and B-cell mitogenic proteins produced by bacteria and African swine fever virus. Indeed, the isoelectric point of p43 is identical to the corresponding proteins secreted by *S. intermedius* (4, 25) and *S. mutans* (eluted after preparative isoelectric focusing between the isoelectric points of 3.9 and 4.2). The immunosuppressive and B-cell mitogenic protein produced by African swine fever virus has a relative molecular mass of 36 kDa and an isoelectric point of 3.89 (1, 3, 14). Although the basic structure of these proteins cannot be inferred from isoelectric points and/or mass data, it should not be very surprising that different microorganisms survive in the host through the production of B-cell mitogenic proteins with a common similar biological structure. Experiments are now in progress in order to test this possibility. In any case, neutralization, by previous elective immunization of these microbial immunosuppressive and B-cell mitogenic substances, seems an appropriate strategy in immunointervention. Indeed, this strategy proved to be appropriate in the protection of mice against *S. mutans* infection (37). Although it is well-known that humoral immunity against unfractionated fungal particles of *C. albicans* has not been shown to provide a protection against infection, we are also now testing the possibility of inducing immunoprotection against this fungus by elective immunization with p43, because this protein seems to be a crucial fungal compound.

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