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Proliferative and cytotoxic responses to mannoproteins of *Candida albicans* by peripheral blood lymphocytes of HIV-infected subjects

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SUMMARY

Mucosal candidiasis is one of the first opportunistic diseases in HIV-infected subjects. In order to understand the relationship between this disease and immunodeficiency to chemically defined, immunodominant *Candida* antigens, a mannoprotein fraction from *C. albicans* cell wall (GMP) was used to analyse proliferative and non-MHC-restricted cytotoxic responses of peripheral blood mononuclear cells (PBMC) from normal and HIV-infected subjects. In the former, GMP induced extensive blastogenesis, generation of powerful cytotoxicity against a tumour cell line (K562), and production of substantial amounts of interferon-gamma (IFN-γ). Cultured PBMC from HIV-infected subjects manifested an early decreased ability for proliferative as well as differentiative cytotoxic responses to the candidal mannoproteins. This inability became clearly evident in subjects with stage III (CDC) of the disease, was total in CDC stage IV and occurred even in some subjects with a normal number of $CD4^+$ cells. Low or absent response to GMP correlated with lack of response to tetanus toxoid. In contrast, both lymphoproliferative and cytotoxic responses to exogeneous IL-2 was highly preserved at all stages of infection. The production of IFN-γ in GMP-stimulated PBMC cultures critically fell to negligible values in most of the subjects in CDC stages II and III. Thus, the lowered or absent cell-mediated immune responses to candidal mannoprotein may be one factor to explain the early, elevated susceptibility of HIV-infected subjects to mucosal candidiasis. This study also shows that our mannoprotein preparation may be used as a probe to detect the overall efficiency of T cell responses in the above subjects.

Keywords HIV infection immune responses *Candida albicans* mannoproteins

INTRODUCTION

Candida albicans is an opportunistic pathogen which possesses distinctive properties of a biological response modifier (BRM) (Cassone *et al.*, 1981; Domer *et al.*, 1988). These BRM properties are in part mediated by mannoproteins, an important antigenic and immunomodulatory constituent of candidal cell wall (Cassone, 1989). A mannoprotein-rich extract was highly effective in activating natural killer (NK) cells and macrophages in mouse peritoneal cavity (Scaringi *et al.*, 1988). It also stimulated lymphoproliferation and induced non-MHC-restricted cytotoxicity in *in vitro* cultured human peripheral blood mononuclear cells (PBMC) (Ausiello *et al.*, 1989; Torosantucci *et al.*, 1990b). Other immunomodulatory effects, including immunosuppressive activities on B and T cells, have also been ascribed to mannoproteins or mannan of *C. albicans* (Domer *et al.*, 1986; Durandy *et al.*, 1987; Carrow & Domer, 1988). Because of the widespread human commensalism of this fungus and consequent natural immunization with it (Cassone, 1989), antigenic extracts of *C. albicans* have been used to assay the state of cell-mediated immunity in HIV-infected subjects. A good correlation was found with diminished reactivity to candidal antigenic extracts and/or tetanus toxoid (TT), and disease progression.

This immunodeficiency, which has been associated clinically with oral and oesophageal candidasis, may be mediated by the functional loss of the T cell receptor (TCR)/CD3 complex (Fauci, 1988; Hofmann *et al.*, 1989; Gruters *et al.*, 1990; Peter *et al.*, 1990). However, this has not been proven, as the candidal antigens tested previously have been poorly defined. As different cell populations might respond to different components of crude candidal extracts, studies with antigens of better-characterized composition should be made.

It is well demonstrated that oral and oesophageal candidiasis represent one of the first and more common opportunistic

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Table 1. Clinical data of patients studied

CDC stage	Patient no.	Sex	Age (years)	Risk category	$CD4^+/\mu l$
II	1	M	31	Former i.v. drug user	561
II	2	F	33	Former i.v. drug user	600
II	3	F	29	Former i.v. drug user	691
II	4	M	28	Former i.v. drug user	1106
II	5	M	27	Bisexual	1200
II	6	F	26	Heterosexual	377
II	7	F	26	I.V. drug user	566
II	8	F	30	Heterosexual	410
II	9	M	43	Homosexual	1079
III	1	F	23	Former i.v. drug user	629
III	2	M	30	Bisexual/i.v. drug user	543
III	3	M	33	Former i.v. drug user	1044
III	4	M	29	Former i.v. drug user	270
III	5	M	14	Blood product	432
III	6	M	28	Heterosexual	347
III	7	M	31	Bisexual/Former i.v. drug user	240
III	8	M	25	Former i.v. drug user	622
III	9	M	31	Homosexual	337
III	10	F	27	Former i.v. drug user	234
III	11	F	33	Former i.v. drug user	532
III	12	M	26	Former i.v. drug user	410
III	13	M	49	Heterosexual	527
III	14	M	26	Former i.v. drug user	900
III	15	F	23	Former i.v. drug user	1200
III	16	M	25	Former i.v. drug user	900
IV.C1	1	M	28	Former i.v. drug user	30
IV.C2	2	M	28	I.v. drug user	263
IV.C2	3	M	30	Former i.v. drug user	100
IV.C2	4	M	33	Heterosexual	462
IV.C1	5	M	28	Former i.v. drug user	200
IV.C1	6	M	29	Former i.v. drug user	357
IV.C1	7	M	37	Former i.v. drug user	166
IV.C2	8	M	38	Homosexual	315
IV.C2	9	M	49	Heterosexual	367
IV.C2	10	F	25	Heterosexual	340
IV.C1	11	F	35	Blood product	101

infection in AIDS patients (Klein *et al.*, 1984; Holmberg & Meyer, 1986; Brawner & Cutler, 1989; Dalgleish & Malkovsky, 1989). It would be important to know at which stage of infection cell-mediated responses to well-defined immunodominant antigens of Candida surface are impaired.

We have purified and characterized a mannoprotein fraction of *C. albicans* which is capable of inducing lymphoproliferation, IL-2 and interferon-gamma (IFN-γ) production, and generation of wide-spectrum cytotoxic effector cells in cultures of human PBMC from all healthy donors tested (Ausiello *et al.*, 1989; Torosanctucci *et al.*, 1990b). Here we report the results of the proliferative and cytotoxic responses of PBMC from subjects at different stages of HIV infection, as defined by the Centers for Disease Control (CDC), to the mannoprotein fraction. TT- or IL-2-induced cell-mediated immune responses were also studied in comparison, in order to evaluate the potential role of purified mannoprotein antigens for early diagnosis of immune deficiency and possible early chemotherapeutic treatments.

SUBJECTS AND METHODS

Patients

Thirty-six subjects (25 men and 11 women) with HIV-1 infection were subgrouped according to their clinical stage (Centers for Disease Control, 1987). The number of subjects in each stage and their clinical characteristics are given in Table 1. Mean age was 30 years (range 14–49). All stage IV patients were under treatment with AZT for at least 6 months, and two of them (patients 1 and 7) were affected by oral candidiasis. In studies with PBMC, blood samples from age- and sex-matched healthy donors were used as controls.

Diagnosis of HIV infection was made by ELISA and confirmed by Western blot (Du Pont de Nemours, Bruxelles, Belgium).

Candida antigen

GMP is a mannoprotein-rich extract from the cell wall of the

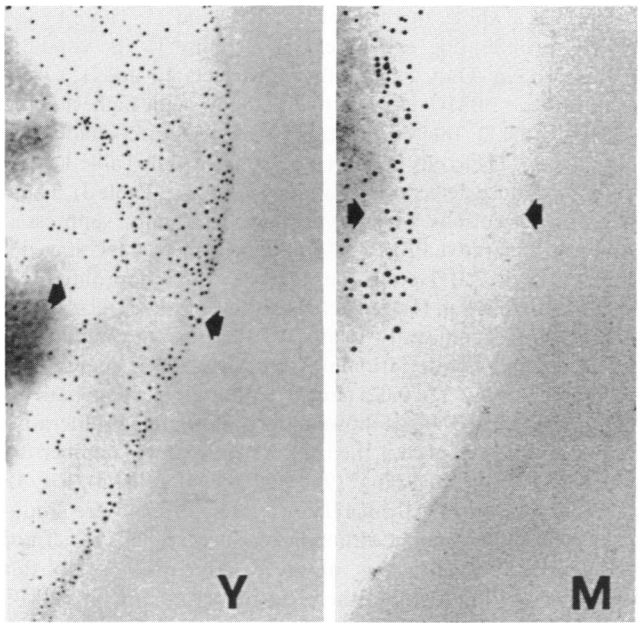

Fig. 1. Electron microscopic visualization by immunogold cytochemistry of GMP constitutents in section of yeast (Y) or mycelial (M) cells of *C. albicans*. Head arrows indicate the cell wall. (For technical details, see Cassone, 1989.)

yeast form *C. albicans* (strain BP). The methods for its preparation and chemical characterization have been described elsewhere (Scaringi *et al.*, 1988; Cassone, 1989; Ausiello *et al.*, 1989; Torosantucci *et al.*, 1990a). In the experiments reported here, a GMP preparation was used containing >80% mannan (detected as mannose in gas-liquid chromatography), and 8·5% protein (Torosantucci *et al.*, 1990a). Mannan and proteins of this extract are present in the form of distinct mannoprotein constituents, mostly of high mol. wt (>200 kD), as detected by SDS–PAGE 5–10% gel gradients and stained with concanavalin A (ConA)/peroxidase or in immunoblots with mAbAF1, a murine monoclonal antibody raised against GMP (Cassone *et al.*, 1988; Torosantucci *et al.*, 1990a). The localization of mAbAF1-reactive mannoprotein constituents on the cell wall of *C. albicans* has been studied with immunogold electron microscope cytochemistry (Cassone, 1989) and is shown in Fig. 1. mAbAF1-reactive epitopes of GMP span the entire wall in the yeast form, but are present only in the inner layers of the mycelial form (see also Cassone *et al.*, 1988; Torosantucci *et al.*, 1990a). The preparation was negative at the *Limulus* lysate gelification test for endotoxin contamination.

Other stimulants

Tetanus toxoid was kindly provided by Wyeth (Marietta, PA). It was used at a final dilution of 1·5 LF/well.

Recombinant IL-2 (rIL-2) (10^7 U/mg) was obtained from Biogen (Geneva, Switzerland).

Lymphocytes

$CD4^+$ lymphocytes were detected by direct immunofluorescence with FITC-conjugated OKT4 (Ortho, Raritan, NJ) using a laser analyser (Ortho Cytoron). The absolute numbers of $CD4^+$ cells were calculated by leucocyte differential count.

PBMC preparation and proliferation assay

Heparinized venous peripheral blood samples were obtained from HIV-1-infected subjects or healthy donors. PBMC were isolated by centrifugation on density gradient (Lymphoprep; Nyegaard, Oslo, Norway), washed in phosphate-buffed saline (PBS) and resuspended in RPMI 1640 medium (GIBCO, Grand Island, NY) supplemented with 5% pooled human AB serum and antibiotics (penicillin 100 U/ml, streptomycin 0·1 mg/ml: GIBCO). Both at this stage and after culture with the various stimulants (see below), cell viability was assessed by the dye exclusion method: >95% of cells were viable in each experiment performed, regardless of the source of the blood sample (control or HIV-infected subject). PBMC (1×10^6) in 0·2 ml of complete medium in 96 flat-bottomed microwell trays (Nunc, Glostrup, Denmark), were cultured with GMP (final concentration 50 μg/ml), TT (final concentration 1·5 LF/ml), or rIL-2 (final concentration 100 U/ml) in triplicate. Plates were incubated in 5% CO_2 at 37°C and harvested on day 7 of culture, according to previously established optimal conditions (Ausiello *et al.*, 1989; Torosantucci *et al.*, 1990b).

Eighteen hours before harvesting, radiolabelled thymidine (Amersham International, Amersham, UK) was added at a final concentration of 0·5 μCi/well. Results were expressed as ct/min (the ^{3}H-thymidine incorporation of unstimulated wells, usually less than 1000 ct/min, was subtracted to the ^{3}H-thymidine incorporation of the test sample).

Generation of cytotoxic effector cells

PBMC were incubated at a concentration of 1×10^6/ml in 24 flat-bottomed microwell plates (Nunc) in 1 ml in the presence of indicated amount of GMP or rIL-2, for 7 days in 5% CO_2, at 37°C, then harvested, washed in RPMI 1640 and resuspended in complete medium. The human erythroblastoid cell line K562 was used as target. Target cells were labelled with 150 μCi of a Na_2-$^{51}CrO_4$ solution (0·1 mCi; specific activity 298·08 mCi/ml) in a final volume of 0·4 ml for 1 h at 37°C. After labelling, the target cells were washed again, resuspended in RPMI 1640 and incubated for 30 min at 37°C. Cytotoxicity assays were performed in round-bottomed, 96-microwell plates, as already reported (Ausiello *et al.*, 1989). Briefly, 5×10^3 target cells were added to suspension of effector cells at three different concentrations, in a total volume of 0·1 ml, in triplicate. Plates were then centrifuged and incubated at 37°C in 5% CO_2 for 4 h, then centrifuged again, and supernatants harvested by a supernatant collection system (Skatron, Oslo, Norway). Radioactivity was assessed by a gamma counter (Compugamma; LKB, Pharmacia, Uppsala, Sweden) and the percentage of specific lysis was calculated as follows:

$$\frac{\text{Experimental} - \text{Spontaneous release}}{\text{Maximum} - \text{Spontaneous release}} \times 100$$

where the spontaneous release was that determined in the absence of effector cells and the maximum release was the total ct/min from artificially lysed cultures.

The values are reported as mean of triplicate samples for each effector:target cell ratio. Standard errors, which never exceeded 5%, have been omitted.

IFN-γ production

IFN-γ production was determined by a radioimmunosorbent assay (Centocor, Malven, PA) on supernatants collected after

7 days of cultures of PBMC stimulated with the indicated concentrations of GMP and rIL-2.

Statistical analysis

Comparisons amongst the different groups of HIV-infected or healthy subjects were performed by the non-parametric Mann–Whitney *U*-test. Comparisons between the means of cytotoxicity were done with Student's *t*-test. Associations between lymphoproliferative responses to GMP and TT and the number of $CD4^+$ cells were evaluated by Pearson's correlation coefficient.

RESULTS

Proliferative response to mannoprotein extract, TT and IL-2

PBMC from 20 healthy donors and 36 subjects at different stages of HIV infection were assayed for their proliferative responses to the mannoprotein extract of *C. albicans*. Several subjects from each of the HIV-infected groups were also tested for response to the TT and IL-2 (Table 2). As shown in Fig. 2, the PBMC from HIV-infected subjects had a markedly decreased proliferative response to GMP, compared with the response of PBMC from healthy controls. The decrease of GMP-induced lymphoproliferation appeared to parallel the stage of infection; in fact, PBMC from CDC stage IV subjects responded lower ($P<0.05$) than those from CDC stage III, and the response of these latter was significantly lower ($P<0.01$) than that of CDC stage II subjects. The median value of lymphoproliferation in the PBMC from this latter group also was lower than median PBMC proliferation of control subjects, although the difference did not reach the statistical significance. Despite the low proliferation of PBMC from CDC stage III subjects as a whole, three subjects (12, 14 and 15; Table 1) had PBMC proliferating extensively in response to the candidal mannoprotein. Only two of them (subjects 12 and 15) were examined for PBMC response to TT, that was the highest of the group (Table 2). Interestingly, one of these subjects had a low number of $CD4^+$ cells (subject 12; 410 $CD4^+/\mu l$; Table 1), and did not produce detectable quantities of IFN-γ (Table 3). There were no exceptions to the very low or totally suppressed lymphoproliferative PBMC response to GMP by CDC stage IV subjects (Fig. 2). Overall, the degree of lymphoproliferative response to GMP in HIV-infected subjects of the CDC stages II and III was significantly related to the absolute number of $CD4^+$ lymphocytes, although the correlation degree was moderate ($r=0.5$, $P<0.02$) (Fig. 3). Noticeably, some patients of the above CDC stages showed low or negligible proliferative response to GMP even if their $CD4^+$ lymphocyte number fell well within the normal range (900 ± 210 $CD4^+$ cells/μl) (Fig. 3).

When the PBMC from HIV-infected subjects were stimulated with TT, a similar, although not overlapping, pattern of proliferative response was observed. The response to this antigen was clearly diminished, or almost absent, in PBMC from CDC stages III or stage IV, respectively (Table 2). Responses to GMP and TT were highly correlated ($r=0.85$, $P<0.001$). In contrast, all HIV-infected subjects responded to stimulation of their PBMC proliferation with IL-2, and no statistically significant difference in this proliferative response was noticed between the controls and CDC stage IV subjects (Table 2). However, PBMC from subjects at CDC stages II or III were significantly less responsive to IL-2 than those from controls or those from CDC stage IV subjects (Table 2). The elevated level of lymphoproliferative response shown by CDC stage IV patients to stimulation with IL-2 demonstrates that the

Table 2. Lymphoproliferative responses* of PBMC from HIV-infected subjects to tetanus toxoid (TT) and IL-2†

CDC stage II			CDC stage III			CDC stage IV		
Patient no.	TT	IL-2	Patient no.	TT	IL-2	Patient no.	TT	IL-2
1	ND	4·1	1	0·1	27·0	1	0·2	13·2
2	6·0	7·0	2	0·0	39·5	2	ND	7·6
3	2·1	10·0	3	1·9	2·1	3	ND	55·6
4	35·0	49·3	4	0·0	49·0	4	1·7	36·8
5	0·4	23·8	5	1·6	31·8	5	ND	44·0
6	ND	ND	6	ND	1·8	6	0·0	38·5
7	7·5	10·5	7	0·5	2·0	7	0·0	60·6
8	84·3	72·1	8	0·0	13·2	8	0·0	28·2
9	23·5	40·0	9	4·5	23·5	9	ND	94·3
			10	1·2	56·5	10	ND	82·0
			11	8·0	5·2	11	ND	70·0
			12	9·0	16·5			
			13	0·0	29·3			
			14	ND	20·0			
			15	79·5	86·5			
			16	1·7	26·0			
Mean ± s.e.m.	22·7 ± 10·3	27·1 ± 8·6		7·71 ± 5·58	26·87 ± 5·7		0·38 ± 0·3	48·26 ± 8·2

*Assessed as ^3H Thymidine incorporation (ct/min $\times 10^{-3}$) in a standard assay.

†Cultures of PBMC from 10 normal subjects (HIV seronegative) stimulated *in vitro* with TT or IL-2 had lymphoproliferative responses of $21·6\pm8·5$ and $43·9\pm10·0$, respectively.

ND, not determined.

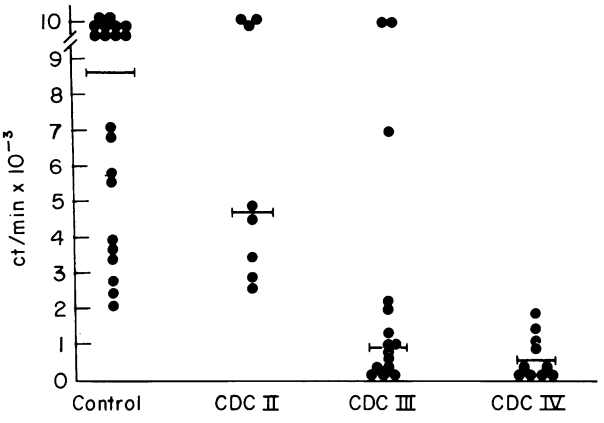

Fig. 2. Lymphocyte proliferative response to mannoprotein (GMP) extract of *C. albicans*. PBMC (10^5 wells) from controls (HIV negative, healthy subjects) or HIV-infected subjects (at the indicated stage of infection) were incubated with GMP (50 μg/ml), and ^{3}H-thymidine incorporation was determined on day 7 of culture. Statistically significant differences exist between control (and CDD stage II) and CDC stage III ($P<0.01$) as well as between and CDC stages III and stage IV ($P<0.05$) according to the Mann–Whitney U-test. Cumulating all data of responses at stages II+III, a statistically significant difference ($P<0.05$) also exists between these subjects and healthy controls. The lines indicate the median values.

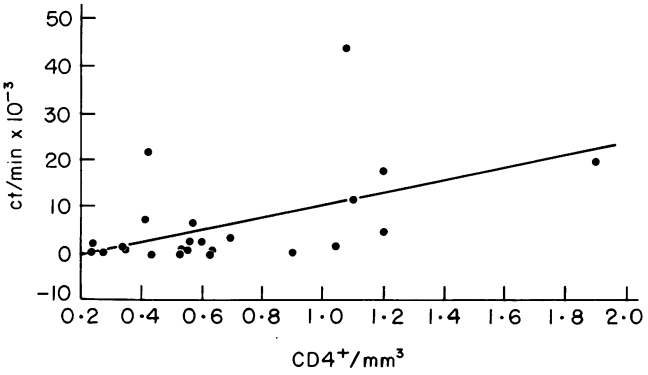

Fig. 3. Correlation between the proliferative response to GMP and the absolute number of $CD4^+$ lymphocytes. Lymphoproliferation was assessed by ^{3}H-thymidine incorporation in a standard assay.

lack of PBMC response to the antigenic stimulants (GMP and TT) was rather specific and was not due to generic metabolic defects of T cells in HIV-infected subjects. This conclusion was also confirmed by the experiments on cytotoxicity generation described below.

Generation of anti-K562 cytotoxic activity by GMP and IL-2

One remarkable property of the GMP extract is its ability to induce non MHC-restricted, LAK-like anti-tumour cytotoxicity in PBMC from healthy donors (Ausiello *et al.*, 1989); we therefore tested the PBMC from HIV-infected subjects for their capacity of killing a usual target of non-MHC-restricted cytotoxicity such as the K562 cells. As a comparison, we also examined whether the response to a powerful activator of this

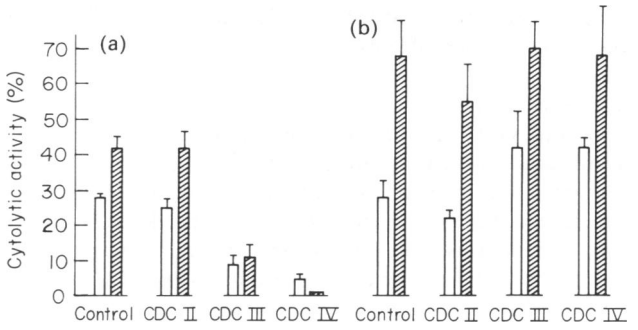

Fig. 4. Generation of cytolytic activity in cultures of PBMC from control and HIV-infected subjects at different stage of infection following stimulation with GMP (a) or IL-2 (b). A statistically significant difference ($P<0.01$) exists between the cytotoxic response of PBMC from CDC stage II and PBMC from CDC stage III subjects in GMP-stimulated, but not in IL-2-stimulated cultures. Results are expressed as percentage of cytolytic activity at E:T ratio of 10:1 (□) and 30:1 (■).

Table 3. Production of IFN-γ (U/ml) by PBMC of HIV-infected subjects (CDC stages II and III) in response to GMP or IL-2 stimulation

CDC stage II			CDC stage III		
Patient no.	GMP	IL-2	Patient no.	GMP	IL-2
2	8	14	1	76	355
3	5	224	2	4	25
4	0	480	3	2	ND
5	2	855	7	7	57
6	8	40	8	12	80
			9	<2	196
			10	162	10
			11	0	180
			12	<2	30
			13	<2	<2
			15	220	160
			16	8	120

The production of IFN-γ in both GMP- and IL-2-stimulated PBMC cultures of 10 control subjects was always > 100 U/ml.
ND, not done.

kind of cytotoxicity, i.e. IL-2, was preserved in the PBMC from HIV-infected subjects. The result of these experiments are shown in Fig. 4. Mannoprotein-induced, anti-K562 cytotoxicity was generated in PBMC from CDC stage II subjects as efficiently as it was in controls. In contrast, PBMC from subsequent stages of infection were almost refractory to GMP induction of cytotoxicity, in particular no anti-K562 activity was generated in PBMC from CDC stage IV subjects. When induced to cytotoxicity generation by IL-2, efficient, high-level anti-K562 lytic activity was observed in cultures of PBMC from subjects at each stage of HIV-infection (Fig. 4b). In particular, the subjects belonging to CDC stage IV did not show a

decreased response to cytotoxicity generation by IL-2, compared with all other HIV-uninfected or infected subjects.

Production of IFN-γ by PBMC stimulated with GMP or IL-2

Since the GMP extract is a powerful inducer of IFN-γ production by PBMC of healthy subjects (Ausiello *et al.*, 1989), we wanted to compare IFN production following GMP or IL-2 stimulation in HIV-infected subjects. We particularly investigated the subjects with low but still detectable responses to GMP as those belonging to CDC groups II and III.

Table 3 shows that, with few exceptions (e.g. subjects 1, 10 and 15, CDC stage III), the PBMC of HIV-infected subjects produced < 10 U of IFN-γ after GMP stimulation, and most of them did not produce any detectable amount of the cytokine. In contrast, PBMC from three out of five CDC stage II subjects and those of five out of 11 CDC stage III subjects produced elevated amounts (> 100 U) of IFN-γ after stimulation with IL-2. Interestingly, of the two patients (10 and 15) whose cells produced more IFN-γ after stimulation with GMP than after stimulation with IL-2 (Table 3), one (patient 10) had a low number of CD4+ cells (Table 1) and her PBMC proliferated extensively in response to IL-2, but not in response to GMP. Only few subjects in CDC stage IV were studied for IFN-γ production in PBMC cultures stimulated with GMP or IL-2. The production of this cytokine in these subjects was very low or absent regardless of the stimulant used (data not shown).

DISCUSSION

We have demonstrated that PBMC from HIV-infected subjects manifest an early loss of proliferative as well as differentiative cytotoxic responses to a mannoprotein-rich extract of *C. albicans*. As emphasized by several investigators (Hofmann *et al.*, 1989; Gruters *et al.*, 1990; Peter *et al.*, 1990), infection with HIV brings about a marked depression of T cell responses mediated through TCR–CD3, which seems to precede the hallmark of HIV pathology, i.e. the diminution of CD4+ cells (Fauci, 1988). In this context, the response to microbial antigens was found to be depressed with progression of the infection (Fauci, 1988; Hofmann *et al.*, 1989). In addition to TT, crude antigenic extracts or even whole Candida cells have been used in HIV-infected subjects (Hofmann *et al.*, 1989; Heagy *et al.*, 1989; Pedersen *et al.*, 1989). These kind of candidal extracts, besides being chemically undefined and of highly variable response, may contain fractions which are by themselves suppressive of the antigenic response (Domer *et al.*, 1986; Carrow & Domer, 1988) or constituents that act more as polyclonal agents than as antigens (Tollemar, Ringdèn & Holmberg, 1989). These drawbacks have been eliminated in this investigation, as the microbial extract used here (GMP) is chemically well defined and molecularly characterized (Scaringi *et al.*, 1988; Ausiello *et al.*, 1989). It is devoid of suppressive activity and has been shown to induce antigenic stimulation of lymphoproliferation of PBMC from healthy subjects (Torosantucci *et al.*, 1990b). The extract is made of about 80% of high molecular weight mannoproteins and its small contaminating RNA fraction was devoid of any immunological influence (Torosantucci *et al.*, 1990b). The existence of a significant correlation between the proliferative responses to GMP and those to TT noticed in this study strengthens the previous evidence for the antigenic, not polyclonal nature of the lymphoproliferation induced by GMP and distinguishes between this complex and other chemically similar materials, acting as polyclonal stimulators (Tollemar *et al.*, 1989).

Although our data do not rule out that at least some of the fully asymptomatic HIV+ subjects may have a reduced PBMC proliferation in response to GMP (see the difference in the median values of lymphoproliferation between control and CDC stage II subjects, Fig. 2), clear-cut deficiency in the proliferative response to the antigen becomes evident in CDC stage III subjects. The overall deficiency in the response to GMP by CDC stages II + III subjects correlated moderately with the number of CD4+ cells, and some of the proliferative responses to GMP were markedly low even in the presence of a normal number of CD4+ lymphocytes. Conversely, a low number of CD4+ cells in these subjects did not preclude a high proliferative response of the PBMC from some of them to candidal mannoprotein as well as to other microbial antigens (Fig. 3, Table 2). All this is in agreement with the concept of selective functional deletions of antigen-specific T cells, already demonstrated *in vitro* in TT-specific T cell responses with the progression of HIV infection (Manca, Habeshaw & Dalgleish, 1990). Our data support the concept of T cell-specific immunosuppression, provoked by HIV or its products (gp120) in asymptomatic subjects, as the early hallmark of HIV disease (Fauci, 1988; Via *et al.*, 1990).

PBMC from HIV-infected subjects also showed a clearly diminished ability to mount a cytotoxic, anti-K562 response on stimulation with GMP. Interestingly, the loss of cytotoxicity induction by GMP in CDC stage IV subjects is not due to a total lack of NK cells, which are advocated to be the precursors of GMP-activated killer cells (Ausiello *et al.*, 1989), as NK activity was totally (CDC stage III) or partially (CDC stage IV) preserved in our HIV-infected subjects (Fontana *et al.*, 1986; Sirianni, Tagliaferi & Aiuti, 1990; other data not shown). The fall of proliferative differentiative responses to antigen stimulation may cause the inability of PBMC from HIV-infected subjects to produce one or more cytokines responsible for the complex cascade of events leading to proliferation/differentiation of cytotoxic effector cells (Fauci, 1988; Via *et al.*, 1990). One such putative cytokine is IL-2, the production of which has been demonstrated to be diminished in PBMC from HIV-infected subjects stimulated by various inducers (Heagy *et al.*, 1989; Via *et al.*, 1990). The IL-2 production was not measured here, but we have shown that HIV-infected subjects have PBMC with highly preserved responsiveness to exogenous IL-2, that is not inhibited by HIV or gp120 (Manca *et al.*, 1990). IL-2-induced proliferation and K562 cytotoxicity were markedly elevated also in those subjects in CDC stage IV who showed very low numbers of CD4+ cells. This may also be explained by the presence of normal number and functions of CD8+ T cells (data not shown).

Previously, we showed that IFN-γ is produced abundantly (100 to > 1000 U/ml) by PBMC of normal subjects cultured in the presence of whole Candida or GMP (Ausiello *et al.*, 1989). However, most of our subjects at the critical stage of the immune response to GMP (CDC stage III) did not produce detectable IFN-γ. This is in keeping with previous results by others (Heagy *et al.*, 1989; Via *et al.*, 1990) using other stimulants. Heagy *et al.* (1989) have recently shown that IFN-γ enhances *in vitro* the proliferative response to whole Candida cells, but only when CD4+ cells are not critically diminished. In one of our CDC

stage III subjects, an appreciable quantity of IFN-γ was produced upon GMP stimulation, but the number of $CD4^+$ cells was low, and no PBMC proliferation was induced by GMP (patient 10, Tables 1–3). Interestingly, the PBMC of this subject produced a low, if not insignificant amount of IFN-γ following stimulation with IL-2. It is clear that marked individual variations in both lymphoproliferation and cytokine production are present among subjects in CDC stage III group, regardless of the nature of the PBMC stimulant (microbial antigen or IL-2), and it is possible that this clinical group is largely heterogeneous as far as functional lymphocyte responses are concerned.

Here we have demonstrated that immune responses to mannoproteins of *C. albicans* are rather early imparied in HIV-infected subjects, an impairment which largely precludes and probably explains the elevated susceptibility of these subjects to mucosal candidiasis (Klein *et al.*, 1984; Holmberg & Meyer, 1986).

The data also suggest that our antigenic preparation (GMP) may be employed as a probe to detect the overall efficiency of T cell responses in the above subjects, at least as satisfactorily as other widely used microbial antigens, e.g. TT. Candidal mannoproteins are expressed on the cell surface of this human commensal micro-organism, and the immune response to them, unlike that to the TT, does not depend on artificial vaccination.

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