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Two novel cationic staphylococcal proteins induce IL-2 secretion, proliferation and immunoglobulin synthesis in peripheral blood mononuclear cells (PBMC) of both healthy controls and patients with common variable immunodeficiency (CVID)

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SUMMARY

Two cationic proteins, a neutral phosphatase (NP-tase) and a 70-kD protein (p70) were isolated from *Staphylococcus aureus* by ion exchange chromatography. We compared their properties to those of the well established B cell mitogen of whole, fixed *Staph. aureus* strain Cowan I cells (SAC). Both purified proteins were able to induce immunoglobulin synthesis in PBMC cultures of healthy donors. NP-tase and p70 also induced immunoglobulin synthesis of PBMC from those patients with CVID who were also responsive to SAC plus IL-2 stimulation. Immunoglobulin synthesis in response to NP-tase and to p70 was time- and dose-dependent and could be inhibited by addition of specific antibodies against the proteins. In contrast to SAC, no addition of exogenous IL-2 was necessary to obtain maximal immunoglobulin synthesis induced by NP-tase or p70. However, neither protein was able to induce immunoglobulin synthesis in B cell-enriched cultures. High amounts of IL-2 were found in supernatants of PBMC from healthy donors following stimulation with low concentrations of NP-tase or p70, and this was associated with vigorous lymphocyte proliferation. Both proteins behave like typical antigens, and not like lectins or superantigens, since an NP-tase-stimulated T cell line showed an antigen-specific, MHC-restricted secondary response. In addition, no preferential T cell receptor Vβ chain usage was found with eight Vβ-specific MoAb. It is likely that the two proteins induce antigen-specific T cell activation, which is then followed by polyclonal activation of B cells via CD40 receptors and cytokine release

Keywords staphylococcal proteins immunoglobulin synthesis IL-2 synthesis B cells CVID

INTRODUCTION

Recently two novel cationic proteins, neutral phosphatase (NP-tase) and 70-kD protein (p70), were purified from the cell surface of the *Staphylococcus aureus* strain Wood 46 [1,2]; neither protein had close similarity to other bacterial proteins with regard to their N-terminal amino acid sequences. NP-tase and p70 bind to human polyclonal IgG (intact and $F(ab')_2$ fragments), but not to Fc fragments of IgG [2]. These properties aroused our interest in the B cell mitogenic potential of NP-tase and p70 and their use in the diagnostic analysis of primary antibody deficiencies, particularly in comparison with the stimulatory effect of whole *Staph. aureus* strain Cowan I (SAC) bacteria. SAC is a potent B cell mitogen which activates B cells, independent of T cells, and induces proliferation and differentiation into immunoglobulin-secreting cells, although IL-2 must be added as a growth factor [3,4]. Purified T cell cultures do not proliferate in response to SAC [5,6]. In this study we analysed NP-tase- and p70-induced immunoglobulin synthesis, T cell proliferation and IL-2 production by PBMC of healthy donors and CVID patients.

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PATIENTS AND METHODS

Patients and controls

Eleven patients with CVID, as defined by the WHO classification [7], and 10 healthy controls were studied. CVID patients and controls were within the same age ranges. The mean age of CVID patients was 39·9 years. CVID patients were regularly

followed up in the clinical immunology out-patient clinic of the Freiburg University Hospital; five had been classified previously into group A, one into group B and five into group C according to the pattern of immunoglobulin production with SAC [3]. All patients received regular i.v. gammaglobulin replacement therapy (Sandoglobin, Polyglobin or Intraglobin; 300 mg/kg body weight every 4–6 weeks), assuring serum IgG levels above 4 g/l. All patients were free of serious infection at the time of testing.

Cell preparation and culture conditions

PBMC were obtained as follows: peripheral blood (40 ml) was defibrinated by gentle rotation in sterile Erlenmeyer flasks containing glass beads. The defibrinated blood was mixed with an equal volume of RPMI 1640 medium (Biochrom, Berlin, Germany), layered onto Ficoll–Hypaque and centrifuged at 900 ***g*** for 20 min. The cells harvested from the interphase were washed twice in RPMI 1640 (10 min at 300 ***g***), layered onto fetal calf serum (FCS) and sedimented by centrifugation (10 min at 300 ***g***). The last step was performed to reduce the amount of cell surface-bound human immunoglobulin. Subsequently, the cells were washed and adjusted to a concentration of 5×10^6/ml in complete RPMI 1640 medium (supplemented with 10% FCS, penicillin 100 U/ml and streptomycin 100 μg/ml, L-glutamine 2 mM and HEPES 0·01 M). All cell cultures were performed at 37°C in a humidified atmosphere containing 5% CO_2. B cells were enriched from PBMC by rosetting T cells twice with neuramidase-treated sheep erythrocytes and subsequent Ficoll density gradient centrifugation, and contained $< 1\%$ $CD3^+$ T cells.

Antigens

SAC bacteria (Calbiochem, Bad Soden, Germany) were washed twice with RPMI 1640 and used at a final dilution of 1 : 25 000. NP-tase and p70 were isolated from *Staph. aureus* strain Wood-46 as previously reported [1,2]. The presence of toxic shock syndrome toxin 1, staphylococcal enterotoxins A–E, and the exfoliative toxins A and B in the bacterial strain was determined by probing for the presence of the respective genes by polymerase chain reaction (PCR). None of these genes was found in the Wood strain [8]. Briefly, staphylococcal cells were harvested by centrifugation (6000 ***g*** for 30 min), washed with 0·05 M Tris-HCl buffer pH 8·0, centrifuged again and resuspended in 44 ml of 1·0 M KCl in 0·5 M Tris-HCl buffer pH 8·5. A crude surface-bound protein fraction was eluted in the final buffer. After centrifugation (6000 ***g***, 30 min), the supernatants were ultracentrifuged (45 000 rev/min for 14 h) and applied in starting buffer (0·32 M NaCl, 0·03 M phosphate buffer pH 7·0) onto a Mono S HRS/5 cation exchange column equilibrated with starting buffer. After 17 ml of buffer had been passed, a linear gradient up to 1·0 M NaCl in starting buffer was run. NP-tase eluted at 0·58 M NaCl and p70 eluted at 0·7 M NaCl. Total protein concentrations were determined with the protein assay reagent (SCA, Pierce, Weiskirchen, Germany).

Anti-NP-tase and anti-p70 antibody preparation

Rabbits were immunized with NP-tase or p70 protein in Freund's complete adjuvant (FCA). Anti-NP-tase or anti-p70 antibodies were prepared by ammonium sulphate precipitation from a single immune serum pool. IgG concentration was 6–8 mg/ml as determined by radial immune precipitation test. Antibody titres in ELISA were positive at IgG concentration of 30 ng/ml.

B cell stimulation assay

PBMC ($1{\cdot}2 \times 10^6$ per assay) were cultured in round-bottomed tissue culture tubes in 1·2 ml complete RPMI 1640 and stimulated with SAC (final dilution 1 : 25 000), NP-tase (0·1–50 μg/ml) or p70 (0·1–40 μg/ml) with and without addition of IL-2 (20 U/ml). To test the antigen specificity, anti-NP-tase antibodies (6·6–100 μg/ml), anti-p70 antibodies (9·6–600 μg/ml) or anti-*Staph. aureus* enterotoxin B antibodies (anti-SEB, dilution 1 : 8; Sigma, Deisenhofen, Germany) were added. Cultures of enriched B cells were set up in 96-well flat-bottomed microplates (Greiner Co., Nürtingen, Germany) using 10^4 cells/well. NP-tase (10 μg/ml), p70 (10 μg/ml) or SAC (1 : 10 000) were added together with IL-2 (20 U/ml). The *de novo* synthesis of IgG and IgM was measured in the supernatants by ELISA [3].

Stimulation of IL-2 synthesis

PBMC ($1{\cdot}5 \times 10^5$) were cultured in a total volume of 200 μl of complete RPMI 1640 in 96-well microplates and stimulated with anti-CD3 MoAb (OKT3, 5 ng/ml; Ortho, Heidelberg, Germany), NP-tase (0·1–10 μg/ml) or p70 (0·1–10 μg/ml). Cumulative IL-2 production was measured in the supernatants after 20, 40 and 70 h using an IL-2-dependent mouse T cell line assay; for details of the colorimetric assay see Mosmann [9].

Assay for proliferative responses

PBMC were cultured as described for the IL-2 assay and stimulated with anti-CD3 antibody (OKT3, 5 ng/ml), NP-tase (0·1–20 μg/ml) or p70 (0·1–20 μg/ml). Anti-SEB antibodies (dilution 1 : 8) were added to PBMC cultures together with 10 μg/ml NP-tase or p70, respectively. After 48 h each well was incubated with 0·5 μCi ^{3}H-thymidine (^{3}H-TdR) for another 24 h until harvest. ^{3}H-TdR uptake was measured by means of a direct beta-counter (Matrix 96; Canberra-Packard, Frankfurt, Germany) and results are given as mean ct/min and s.d.

T cell lines

PBMC (10^6/ml) were stimulated with NP-tase (1 μg/ml) or p70 (1 μg/ml). After 3 days IL-2 (20 U/ml) was added and after 15 days resulting T cell lines were phenotyped and tested for antigen-specific proliferative responses or interferon-gamma (IFN-γ) production.

Proliferative responses of T cell lines

Adherent antigen-presenting cells (APC) were obtained by incubating autologous or allogenic PBMC (4×10^5/200 μl RPMI 1640 plus 10% FCS) in microplates for 2 h at 37°C. Non-adherent cells were removed by washing the plates several times. T cell lines were added at 2×10^4/well alone or with addition of NP-tase (1 μg/ml), p70 (1 μg/ml) and anti-CD3 (10 ng/ml) or SEB (1 ng/ml). Culture supernatants were collected after 48 h and tested for IFN-γ production. Proliferative responses of the cultures were assayed in parallel by a 24-h pulse of ^{3}H-TdR.

IFN-γ ELISA

IFN-γ was tested in culture supernatants by ELISA. 96-well microplates (Greiner) were coated with anti-human IFN-γ MoAb (4 μg/ml PBS, M-700A; Endogen, Boston, MA) over-

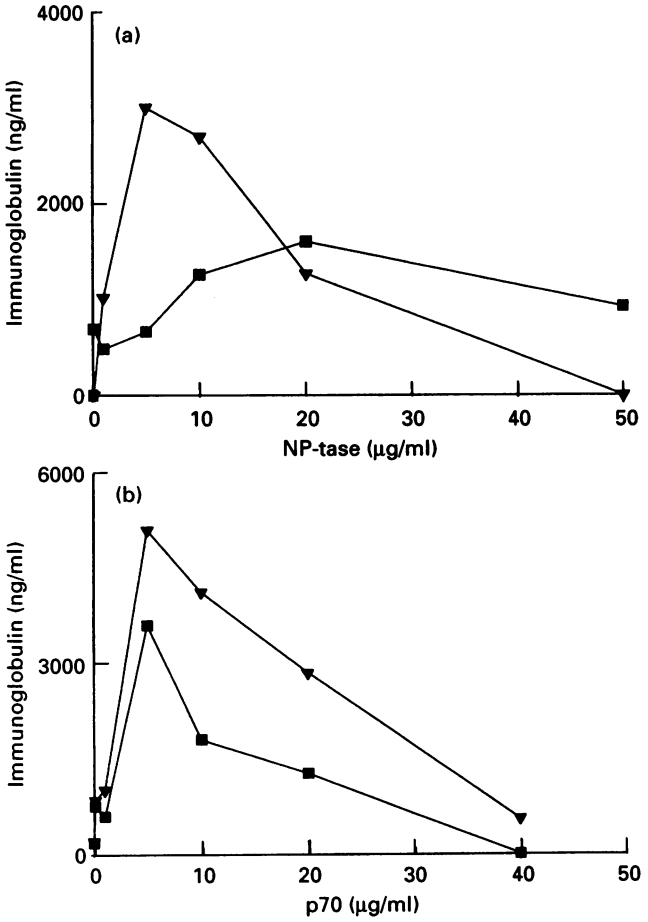

Fig. 1. IgM and IgG synthesis *in vitro* after stimulation with 70-kD (p70) protein or neutral phosphatase (NP-tase). Immunoglobulin synthesis of PBMC of a healthy donor was measured after stimulation for 9 days with different concentrations of NP-tase (a) or p70 (b) by ELISA. One representative experiment out of three is shown. ▼, IgM; ■, IgG.

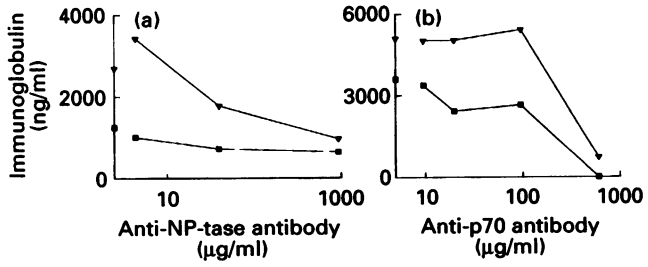

Fig. 2. Inhibition of IgM and IgG synthesis by anti-70-kD protein (p70) antibodies or anti-neutral phosphatase (NP-tase) antibodies. PBMC of a healthy donor were stimulated with NP-tase (10 µg/ml) in the presence of increasing concentrations of anti-NP-tase antibodies (a) or with p70 (5 µg/ml) in the presence of anti-p70 antibodies (b). IgG (■) and IgM (▼) levels were measured in the culture supernatants after 9 days by ELISA. Immunoglobulin production in the absence of antibodies against staphylococcal proteins is indicated by open symbols on the vertical axis.

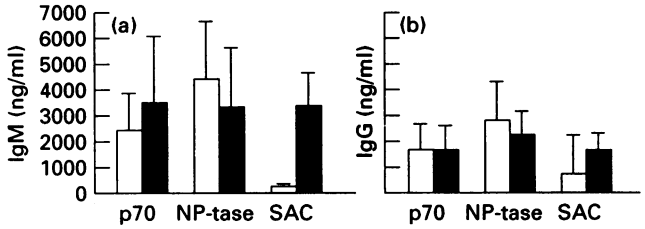

Fig. 3. Immunoglobulin synthesis in response to neutral phosphatase (NP-tase) or 70-kD protein (p70) is not enhanced by exogenous IL-2. PBMC of 10 healthy donors were cultured for 9 days with NP-tase (5 µg/ml), p70 (20 µg/ml) or *Staphylococcus aureus* Cowan I (SAC) (dilution 1 : 25 000) with (■) and without (□) the addition of IL-2 (20 U/ml). (a) IgM and (b) IgG concentrations were measured in the supernatants and given as mean values and s.d.

night at 4°C. Plates were washed with PBS plus 0·05% Tween 20 (Sigma) and blocked with PBS plus 10% FCS for 1 h at room temperature. Undiluted culture supernatants (100 µl) or serial dilutions of human recombinant IFN-γ (Bioferon, Laupheim, Germany) as standard (300–10 000 pg/ml) were incubated for 1 h at room temperature. After washing, 100 µl well of biotinylated anti-human IFN-γ antibody (1 : 2000, M-701; Endogen) were added and the plates incubated for 1 h. Subsequently, plates were washed, streptavidin-conjugated alkaline phosphatase (1 : 1000; Dianova, Hamburg, Germany) was added and incubation continued. After 1 h plates were extensively washed, developed with *p*-nitrophenylphosphate (Sigma) and absorption was measured at 405 nm with a MR5000 reader (Dynatech, Denkendorf, Germany). IFN-γ concentrations were calculated on the basis of the standard curve.

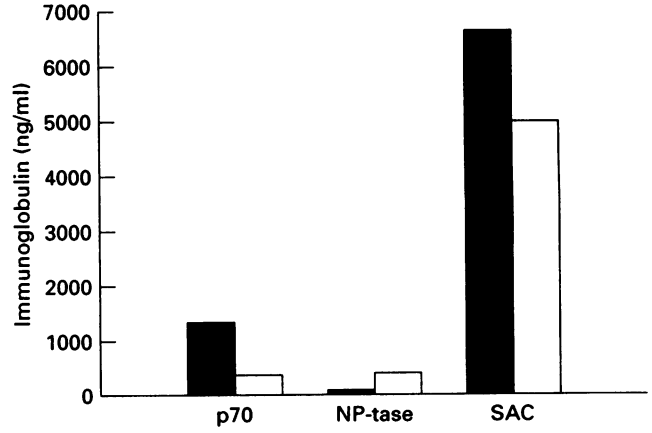

Fig. 4. Enriched B cells do not produce immunoglobulin after stimulation with 70-kD protein (p70) or neutral phosphatase (NP-tase) plus IL-2, but do with *Staphylococcus aureus* Cowan I (SAC) plus IL-2. B cells were enriched by rosetting PBMC with neuraminidase-treated sheep erythrocytes twice. The B cell-enriched population consisting of 65% B cells ($CD19^+$) and less than 1% T cells ($CD3^+$) was cultured for 9 days at 1×10^4/well in the presence of p70 (10 µg/ml), NP-tase (10 µg/ml) or SAC (1 : 10 000) and IL-2 (20 U/ml). IgM (■) and IgG (□) were measured in the culture supernatants by ELISA. A second experiment with B cells from another healthy donor gave similar results (not shown).

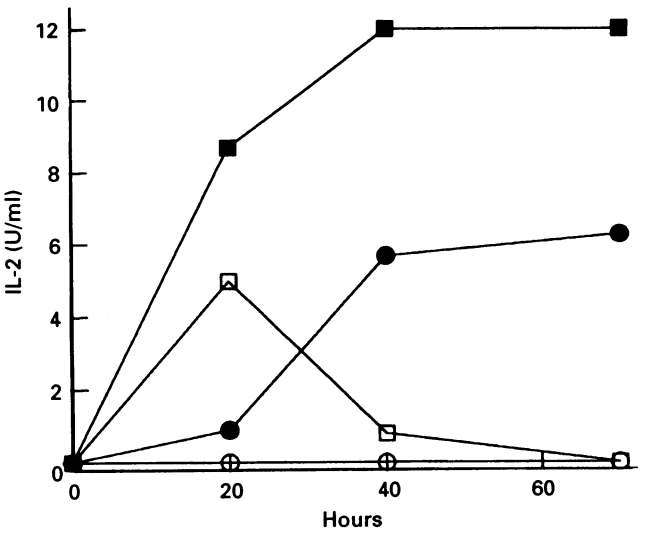

Fig. 5. Induction of IL-2 synthesis by 70-kD protein (p70) and neutral phosphatase (NP-tase). IL-2 was measured in the culture supernatants 20, 40 and 70 h after stimulation of PBMC with p70 (●, 1 μg/ml), NP-tase (■, 1 μg/ml), *Staphylococcus aureus* Cowan I (SAC) (○, dilution 1 : 25 000) or OKT3 (□, 5 ng/ml). One out of two similar experiments is shown.

Phenotyping of PBMC and T cell lines

Phenotyping was performed by flow cytometric analysis with FITC- or PE-conjugated MoAbs specific for CD3, CD4, CD8, CD19 (Dakopatts, Hamburg, Germany) or T cell receptor variable elements Vβ5c, Vβ6, Vβ12 (Biermann, Bad Nauheim, Germany), Vβ2, Vβ8, Vβ13, Vβ17, Vβ19 (Dianova).

RESULTS

Immunoglobulin synthesis in vitro

NP-tase and p70 induced a time- and dose-dependent IgM and IgG synthesis in normal PBMC cultures. The immunoglobulin synthesis peaked on day 12 of culture (not shown). Both proteins induced maximal IgM and IgG synthesis at concentrations of 5–20 μg/ml (Fig. 1a,b). This immunoglobulin synthesis could be specifically inhibited in a dose-dependent manner by addition of antibodies against the proteins (Fig. 2a,b). Anti-p70 antibodies did not inhibit immunoglobulin synthesis after stimulation with NP-tase, and *vice versa* anti-NP-tase antibodies did not have an effect on immunoglobulin production after stimulation with p70. Furthermore, addition of anti-SEB antibodies did not inhibit stimulation of immunoglobulin synthesis by both proteins (data not shown). In clear contrast to SAC stimulation, which requires IL-2 supplementation in order to induce high IgM and IgG production by B cells, addition of IL-2 (20 U/ml) to NP-tase- or p70-stimulated cultures did not increase IgM and IgG synthesis (Fig. 3). However, enriched B cell populations could not be activated to secrete immunoglobulin after stimulation with NP-tase or p70, even after supplementation with IL-2, whereas SAC plus IL-2-triggered B cells showed a vigorous synthesis (Fig. 4).

NP-tase and p70 induce IL-2 synthesis and T cell proliferation

Prompted by the above findings, we investigated the potency of NP-tase and p70 to induce IL-2 synthesis in PBMC cultures from two healthy donors. IL-2 production was observed (with both proteins) at concentrations ranging from 0·1 to 10 μg/ml (results not shown). Using 1 μg/ml of p70 or NP-tase a time-dependent IL-2 synthesis was observed, peaking between 40 and 70 h (Fig. 5). In comparison with OKT3-stimulated lymphocyte cultures, induction of IL-2 production by NP-tase or p70 was higher and more persistent. SAC was unable to stimulate IL-2 synthesis of T cells (Fig. 5), as already known. In concordance with the results on IL-2 production, both NP-tase and p70 induced a vigorous proliferative response of PBMC over a broad range of concentrations (0·1–20 μg/ml; two representative experiments out of three are shown in Table 1). This NP-tase- and p70-induced stimulation could not be inhibited by the addition of anti-SEB antiserum (Table 1, experiment 2).

NP-tase and p70 do not have lectin- or superantigen-like properties

The strong T cell activation induced by NP-tase and p70 prompted us to test whether these proteins exhibit lectin- or superantigen-like properties. Short-term T cell lines were established by stimulation of PBMC with NP-tase or p70 and expanded in the presence of IL-2 for 15 days. The T cell lines obtained were composed of 78% or 80% $CD3^+$, 56% or 60% $CD4^+$ and 20% or 18% $CD8^+$ cells for NP-tase or p70, respectively. An enrichment of particular T cell receptor variable elements, which is typically found after superantigenic stimulation, was not observed with NP-tase- or p70-stimulated T cell lines using eight commercially available MoAbs tested by flow cytometry (results not shown). The T cell lines were subsequently tested for their proliferative and cytokine responses to NP-tase, p70, SEB and anti-CD3 MoAb in the presence of autologous or allogeneic monocytes as APC. Both the NP-tase- and the p70-stimulated T cell lines exhibited a

Table 1. Proliferative responses of PBMC to neutral phosphatase (NP-tase) and 70-kD protein (p70)

Antigen concentration (μg/ml)	Proliferative response (ct/min)	
	p70	NP-tase
Experiment 1		
None	1200	1200
0·5	2990	8110
2·5	5180	8980
7·5	5860	8560
15·0	9150	4740
Experiment 2		
None	3650	3650
0·1	20 460	14 453
1·0	31 110	26 280
5·0	31 570	32 650
10·0	30 670	22 150
10·0 + anti-SEB (1 : 8)	28 370	24 815

PBMC ($1{\cdot}5 \times 10^5$/well) were stimulated with p70 or NP-tase at the concentrations given and ^{3}H-thymidine incorporation was determined after 48 h. For comparison, anti-CD3 (5 ng/ml) stimulation induced 24 920 ct/min in experiment 1.

Table 2. Neutral phosphatase (NP-tase)-specific secondary T cell response

T cell line	Source of monocytes	Proliferative response (ct/min)					IFN-γ production (pg/ml)				
		Medium	NP-tase	p70	Anti-CD3	SEB	Medium	NP-tase	p70	Anti-CD3	SEB
NP-tase	Autologous	4745	8144	3817	11301	12290	366	3384	294	7954	6415
p70	Autologous	3051	3698	3176	12965	11128	152	408	221	2645	2606
NP-tase	Allogeneic	239	1123	1570	9807	9798	430	144	930	6616	4453
p70	Allogeneic	828	1357	1546	11795	10023	330	491	162	2745	1848

T cell lines were obtained by culture of PBMC in the presence of NP-tase or 70-kD protein (p70) (1 μg/ml) and IL-2 (20 U/ml). After 15 days the secondary response of the T cell lines was tested in the presence of autologous or allogeneic monocytes with addition of NP-tase (1 μg/ml), p70 (1 μg/ml), anti-CD3 (10 ng/ml) or SEB (1 ng/ml) and quantified by ^{3}H-thymidine incorporation or IFN-γ production (for details see Patients and Methods). Significant antigen-specific responses are underlined.

considerable degree of autoreactivity, as demonstrated by their proliferative responses to autologous but not allogeneic APC alone (Table 2). This phenomenon is well known for all antigenic stimulations [10]. Despite this autoreactive background response, the NP-tase-stimulated T cell line showed an enhanced proliferation and, even more impressively, a strong IFN-γ production in response to NP-tase. This response was antigen-specific, since p70 was not recognized, and MHC-restricted, since no response occurred with allogeneic APC (Table 2). The p70-stimulated T cell line did not show an antigen-specific response exceeding the autoreactive background. On the other hand, anti-CD3 MoAb and SEB induced a strong response of both T cell lines irrespective of the source of APC, which is in accordance with their mitogenic properties.

Immunoglobulin synthesis in vitro *by PBMC from CVID patients*

Patients with CVID are unable to produce normal amounts of specific immunoglobulin following antigenic stimulation *in vivo*. It was of interest to assess the effect of p70 and NP-tase on IgG and IgM synthesis by PBMC of CVID patients. p70- and NP-tase-stimulated PBMC from healthy individuals and CVID patients yielded the same pattern of *in vitro* IgG and IgM synthesis as previously described with SAC-stimulated cultures [3]. Five CVID patients classified into group A did not secrete immunoglobulin after stimulation with NP-tase or p70, one group B patient showed low IgM but no IgG synthesis, and five patients classified into group C produced normal amounts of IgM and IgG (Fig. 6). Remarkably, two of the latter patients had been classified in group C1 [3] due to their inability to produce immunoglobulin following stimulation with pokeweed mitogen. As observed with normal PBMC, IL-2 did not augment immunoglobulin synthesis of CVID lymphocytes stimulated with p70 or NP-tase (Fig. 6).

DISCUSSION

In this study we investigated the mechanism of the immune response induced by two novel staphylococcal cationic proteins. We could demonstrate that both proteins induce T and B cell responses, but they exhibit properties that are distinct from those of superantigens such as SEB and from those of mitogens like SAC. This is confirmed by several lines of experimental evidence. The *Staphylococcus* strain used to purify NP-tase and p70 is negative for enterotoxin genes [8], excluding the possibility that the results are due to contamination with enterotoxins. The vigorous IL-2 production and T cell proliferation occurring after stimulation of PBMC with NP-tase and p70 could not be inhibited by antibodies to SEB. Using T cell lines, it was shown that NP-tase and probably p70 activate T cells specifically by an

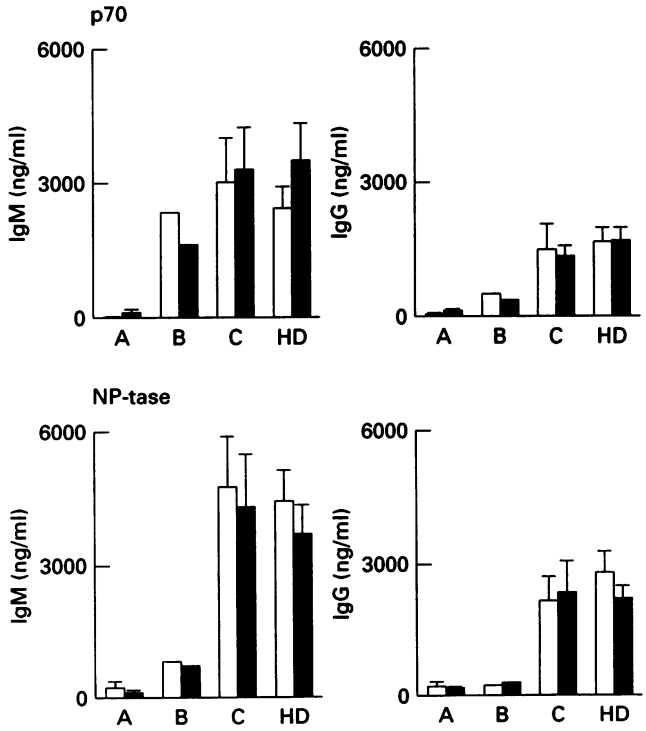

Fig. 6. Immunoglobulin secretion by PBMC of CVID patients upon stimulation with neutral phosphatase (NP-tase) or 70-kD protein (p70). PBMC of 11 CVID patients and 10 healthy controls (HD) were stimulated with NP-tase (5 μg/ml) or p70 (20 μg/ml) with (■) and without (□) the addition of IL-2. Five patients are classified into group A, one patient into group B and five patients into group C (classification according to [3]). IgM and IgG secretion was measured in the supernatants by ELISA. Results are given as means and s.e.m.

MHC-restricted antigen presentation mechanism. For both proteins, NP-tase and p70, lectin- or superantigen-like properties in T cell activation could clearly be excluded, since MHC-unrestricted responses were not observed with these T cell lines. On the other hand, the superantigen SEB induced antigen and MHC haplotype-independent proliferation of T cell lines, as expected [11].

The induction of B lymphocyte responses is a complex process that can be initiated either by T cell-dependent antigens or by T cell-independent mitogens like SAC. SAC induces B cell proliferation by cross-linking of surface immunoglobulin and IL-2 receptor expression. Addition of exogenous IL-2 is followed by B cell differentiation to immunoglobulin-secreting plasma cells [12]. Similarly, the proteins p70 and NP-tase induce differentiation into immunoglobulin-secreting plasma cells when PBMC are used. However, no supplementation with B cell differentiation factors was necessary to obtain maximal immunoglobulin production *in vitro*, and addition of exogenous IL-2 did not further augment immunoglobulin synthesis in this case. In contrast to PBMC, B cell-enriched cultures with less than 1% T cells could not be activated by NP-tase or p70 to secrete immunoglobulin, even after supplementation with IL-2. Taken together, these data imply that, unlike SAC, NP-tase and p70 do not activate B cells directly, presumably due to lack of receptor cross-linking, but induce T cell proliferation and IL-2 secretion. We propose that these activated T cells subsequently induce the stimulation of resting B cells in a non-antigen-specific manner, probably via CD40–gp39 receptor interactions and T cell-derived lymphokines. Since the novel cationic staphylococcal proteins do not show superantigenic properties, their mechanism of inducing B cell activation and differentiation is different from that described for anti-CD3 or superantigen-activated T cells [13,14], most probably involving antigen-specific T cells. Although a high frequency of T cells recognizing staphylococcal antigens may be expected, the exact mode of NP-tase- and p70-induced T cell activation and T–B cell interaction has still to be clarified.

Patients with CVID are unable to produce specific immunoglobulin after antigen contact *in vivo*. In previous studies it was shown that B cells from CVID patients displayed different patterns of IgG and IgM responses following stimulation with SAC plus IL-2 *in vitro*. According to their B cell responses the patients were classified into three groups [3,15]. Patients in group A failed to synthesize IgG or IgM, patients belonging to group B produced only IgM but no IgG, whereas patients in group C showed no defect and produced IgM and IgG normally. The data obtained in this study indicate that the two novel cationic staphylococcal antigens cannot overcome the disturbed T–B cell interaction in CVID with regard to group A and B patients. Most interestingly, both T cell-dependent proteins induced normal immunoglobulin secretion of B cells from two group C1 patients, although pokeweed mitogen was ineffective [3]. Thus, NP-tase and p70 may become valuable tools in the experimental analysis of the defective T–B cell interactions in CVID.

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