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Idiotype-induced T cell stimulation requires antigen presentation in association with HLA-DR molecules

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SUMMARY

An important and yet unresolved question concerns the mode of T cell recognition of idiotypic epitopes on immunoglobulin molecules in humans. Results from murine and human studies show that some idiotype-specific T cells recognize conformational epitopes on immunoglobulin, and such T cells are not MHC-restricted. In the present study T cell stimulation induced by idiotypic determinants on the autologous monoclonal IgG (M-components) from patients with monoclonal gammopathies was studied. In parallel, T cell stimulation in response to a conventional antigen, purified protein derivative, was also examined. It is shown that, as with conventional antigen, idiotype-induced T cell stimulation requires the presence of antigen-presenting cells (APC; monocytes and/or B cells), and is MHC class II (DR)-restricted. B cells, but not monocytes, can present idiotypic determinants to T cells at very low antigen concentrations, while monocytes do so only when antigen is present at high concentrations. Antigen processing and presentation is abrogated by treatment of APC with chloroquine. In conclusion, our study demonstrates that human idiotype-specific T cells recognize processed idiotypic determinants presented by MHC class II (HLA-DR) molecules on APC, and that B cells require about 1000-fold less antigen than monocytes.

Keywords M-component idiotype T cells antigen presentation MHC molecules

INTRODUCTION

The idiotypic network hypothesis, originally proposed by Jerne [1], proposed that interactions between idiotype (Id) and anti-Id determinants select and regulate the available repertoire in the immune system. Participation of T and B lymphocytes in Id-based regulatory systems has been intensively investigated in both normal and disease situations [2–5]. In B cell tumours such as monoclonal gammopathies, idiotypic structures on the variable regions of the immunoglobulin on the malignant B cell surface and produced as a serum M-component may represent a clonal marker, and as such they can act as tumour-specific antigen [3,6,7]. B cells producing anti-Id antibodies and T cells reactive with Id of the autologous M-components have been described in murine myeloma [8–11] and in human multiple myeloma [12–18]. Such an anti-idiotypic immune response has been shown to be important in the regulation of clonal B cell tumour in animals [6,19].

An important and yet unresolved question concerns the mode of T cell recognition of idiotypic determinants on immunoglobulin. Earlier studies of animal and human systems have yielded conflicting results. Id-reactive T cells were shown to bind directly to Id^+ immunoglobulin molecules (conformational epitopes) [10,12,16,20–25], and some of the Id-specific T cells were not MHC-restricted [22,25]. More recently, experiments with murine plasmacytoma have, however, demonstrated that Id-specific $CD4^+$ T cell clones recognize only processed peptides of idiotypic determinants in the context of MHC class II molecules on APC [26,27]. Nevertheless, whether this is true also for human Id immunoglobulin has not been shown.

This study examined the mode of T cell recognition of Id determinants in man. We studied the requirements of molecules and cells involved in T cell activation induced by the autologous $F(ab')_2$ fragments of monoclonal IgG from patients with monoclonal gammopathies. As a control, T cell stimulation induced by a conventional antigen, purified protein derivative (PPD), was examined in parallel. Our results show that APC such as monocytes or B cells, and MHC class II (HLA-DR) molecules were required in Id-induced T cell stimulation. Id-specific T cells recognized only processed idiotypic peptide fragments presented by MHC molecules on APC.

MATERIALS AND METHODS

Patients

The study comprised five patients with monoclonal gammopathies

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of undetermined significance and six patients with multiple myeloma stage I. The diagnostic criteria have been described elsewhere [28,29]. The clinical staging system for multiple myeloma according to Durie & Salmon [30] was used. At the time of study, none of the patients was being treated with immunosuppressive drugs.

Preparation of monoclonal IgG and F(ab′)$_2$ fragments from patients

The procedure was described in detail elsewhere [15]. Briefly, IgG was purified by a MabTrapG-column (Pharmacia, Uppsala, Sweden). The purity of the IgG fraction was confirmed using SDS–PAGE under reducing and non-reducing conditions (Pharmacia Phast System). Isoelectric focusing (Pharmacia Phast system) confirmed that 90–99% of the IgG was monoclonal. F(ab′)$_2$ fragments were prepared by pepsin digestion as described earlier [14,31,32].

Antibodies

For the identification of blood T cells, B cells and monocytes, FITC- or PE-conjugated MoAbs against CD3, CD19 and CD14 (Becton Dickinson Immunocytometry Systems, Mountain View, CA) were used. To study MHC restriction, purified mouse MoAbs against MHC class II molecules HLA-DR (IgG2b; Immunotech, Marseille, France), -DP (IgG1; Becton Dickinson), -DQ (IgG2b; Sigma, St Louis, MO), and class I molecules HLA-ABC (IgG2b; Chemicon International Inc., Temecula, CA), and isotypic control IgG2b (Immunotech) were used.

Flow cytometry analysis

Cells were stained by FITC- and PE-conjugated antibodies and analysed by a FACScan flow cytometer (Becton Dickinson) as described previously [33].

Cell preparation

Heparinized venous blood was diluted twice in PBS and layered onto Ficoll–Paque (Pharmacia) [34]. After centrifugation, interphase cells (peripheral blood mononuclear cells (PBMC)) were collected, washed and resuspended in culture medium (RPMI 1640; GIBCO Ltd, Paisley, UK) supplemented with L-glutamine 4 mM, penicillin 100 U/ml, streptomycin 100 μg/ml, and 10% heat-inactivated (56°C, 30 min) pooled serum from individuals with blood group ABRh$^+$.

Monocytes were depleted of PBMC after incubation with iron powder [35]. This method resulted in peripheral blood lymphocytes (PBL) containing <1% CD14$^+$ cells. Monocytes were enriched from EDTA (0·002%) blood. The cells were isolated by Nycodenz (density 1·006 g/ml) (Nyegaard A/S, Oslo, Norway) gradient centrifugation [36]. Over 90% of the cells in the preparation were CD14$^+$ cells.

B cells were depleted from PBMC by incubating cells with anti-CD19 antibody-coated Dynabeads (M-450; Dynal A.S., Oslo, Norway), at a bead to target cell ratio of 50:1 (negative selection), according to the manufacturer's instructions. More than 95% of CD19$^+$ B cells were depleted. B cells were purified by incubating PBMC with the beads at a bead to target cell ratio of 3:1 (positive selection), and then separated from the beads by incubation with detaching beads (DETACHaBEAD; Dynal A.S.). This resulted in a cell suspension containing >95% CD19$^+$ cells.

T cells were isolated from PBMC after forming rosettes with sheep erythrocytes [37]. The resulting cell suspension contained >90% CD3$^+$ T cells, <0·5% CD14$^+$ monocytes, and <0·4% CD19$^+$ B cells, as assessed by FACScan analysis.

Cell proliferation

A 96-well round-bottomed microtitre plate (Nunc U96; Nunclon, Roskilde, Denmark) was used. Cells (1×10^5/well) were added to each well and incubated with 10 pg/ml to 100 μg/ml of F(ab′)$_2$ fragments of the autologous or isotypic monoclonal IgG for 3–6 days in air with 5% CO_2 at 37°C. Cells incubated with medium only or with PPD (2·5 μg/ml) were used as controls. Eighteen hours before harvest, 1 μCi/well of ^{3}H-thymidine (Amersham Life Science, Aylesbury, UK) was added. The cells were collected using a Skatron combi cell harvester (Skatron A/S, Lier, Norway), and radioactivity was measured in a liquid scintillation counter (LKB1212 Rackbeta; Pharmacia). The results are expressed as mean ct/min of triplicate incubations. The coefficient of variation between triplicate tests was 13·5%.

Cells secreting interferon-gamma

The enzyme-linked immunospot assay for identification of interferon-gamma (IFN-γ)-secreting cells was used as described earlier [17]. Briefly, plates (Millititer-HAM; Millipore Co., Bedford, MA) were coated with a mouse anti-human IFN-γ MoAb (Genzyme Corp., Boston, MA). PBMC (1×10^5/well) were added and incubated with 0·1 pg/ml to 10 μg/ml of F(ab′)$_2$ fragments of the autologous or isotypic monoclonal IgG. Cells incubated with no addition or with PPD (2·5 μg/ml) were used as controls. After incubation for 48 h, cells were detached from plates by washing, and rabbit polyclonal anti-human IFN-γ (Genzyme) was added. After further washing, the spots were developed by sequential incubation with biotinylated anti-rabbit IgG (Vector Labs, Burlingame, CA), avidin-biotin peroxidase complex (ABC Vectastain-Elite Kit; Vector Labs), followed by peroxidase staining, using the substrate 3-amino-9-ethyl-carbazol (Sigma). Spots corresponding to IFN-γ-secreting cells were enumerated under a dissection microscope. The coefficient of variation between triplicate values was 8·5%.

All samples were incubated in duplicate wells. Data are expressed as numbers of IFN-γ-secreting cells/10^5 PBMC. The number of stimulated cytokine-secreting cells is defined as the total number of spots minus the number of spots in cultures with medium only.

Chloroquine treatment

A lysosomotropic agent (chloroquine) was used to inhibit antigen processing and presentation [38,39]. The effect of the drug was evaluated by culturing PBMC in the presence or absence of the autologous or isotypic IgG F(ab′)$_2$ fragments, PPD or concanavalin A (Con A; 20 μg/ml), with or without the addition of chloroquine (25 μM; Sigma). Alternatively, adherent cells, obtained from PBMC incubated on a Petri dish for 30 min at 37°C, were incubated with the antigens in the presence or absence of the drug (200 μM) for 2 h at 37°C and then used as APC.

RESULTS

F(ab′)$_2$ fragment-induced cell stimulation

Incubation of PBMC with F(ab′)$_2$ fragments of the autologous monoclonal IgG resulted in increased IFN-γ-secreting cells. As

exemplified by the experiments in Fig. 1, a biphasic dose-dependent response was noted. Cells were activated by low (1–100 pg/ml) and by high (100 ng/ml to 10 μg/ml) concentrations of the fragments. Cells from some patients were stimulated by isotypic $F(ab')_2$ fragments at high concentrations (1–10 μg/ml) (Fig. 1b). Autologous $F(ab')_2$ fragment-induced IFN-γ-secreting cells were increased in nine of 11 patients analysed in this study.

Cell proliferation induced by the antigens in cultures for 3–6 days was determined, and a 6-day culture was chosen since it was optimal for inducing cell proliferation. In six of nine patients who responded with an increase in the number of IFN-γ-secreting cells, cell stimulation was also noted by the cell proliferation assay. However, the dose–response pattern was different from cytokine secretion. PBMC from four of the six patients proliferated only to high concentrations (10 ng/ml to 10 μg/ml) of the autologous $F(ab')_2$ fragments (Fig. 2a), while cells from the other two patients showed a biphasic dose-dependent response (low dose: 10 pg/ml to 1 ng/ml), similar to IFN-γ secretion (for example, see Fig. 5). In some cases, isotypic $F(ab')_2$ fragments also induced cell proliferation (Fig. 2b).

Based on these results, the six patients who responded with both IFN-γ secretion and cell proliferation were chosen for further studies. These patients also responded to PPD by cell proliferation and cytokine secretion. Although only the results from cell proliferation are presented, all the following experiments were performed by detecting both IFN-γ-secreting cells and cell proliferation, and the results were confirmed by at least three tests.

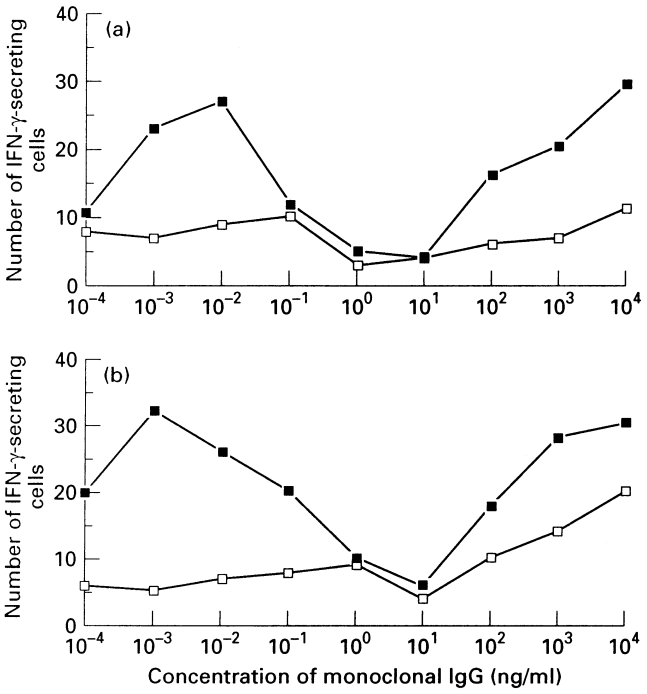

Fig. 1. Stimulated numbers of IFN-γ-secreting cells/10^5 peripheral blood mononuclear cells (PBMC) incubated with $F(ab')_2$ fragments of the autologous (■) or isotypic (□) monoclonal IgG. Results from two patients are shown. (a) A proliferative response only to the autologous monoclonal IgG. (b) Also to the isotypic monoclonal IgG at high antigen concentrations.

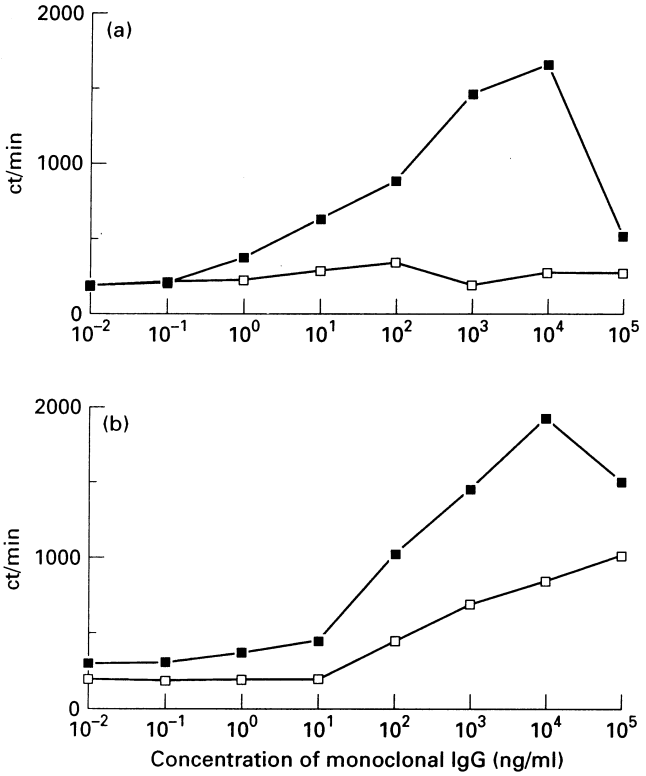

Fig. 2. Cell proliferation in response to $F(ab')_2$ fragments of the autologous (■) or isotypic (□) monoclonal IgG from two patients. Results from two patients are shown (a and b).

Antigen-presenting cells

First, monocytes were depleted from PBMC and the resulting cell population (PBL) was tested for reactivity to the autologous $F(ab')_2$ fragments. As depicted in Fig. 3, PBMC depleted of monocytes (PBL, $CD14^+$ cells < 0·2%), and T cells alone did not respond to the antigen. This finding had been confirmed with cells from three patients that proliferated only to the autologous IgG $F(ab')_2$ fragments. We then tried to reconstitute the system by adding back monocytes. Since our previous study demonstrated that 5% of

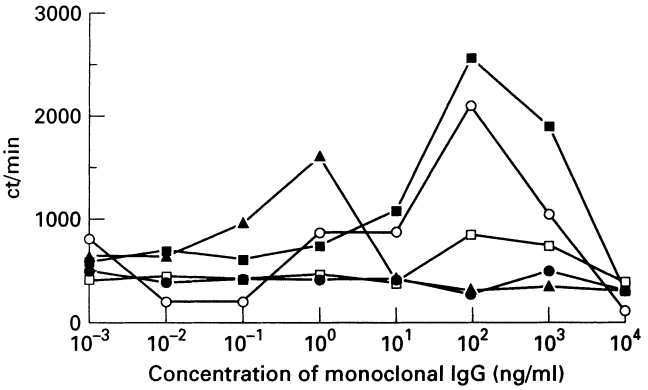

Fig. 3. Proliferation of peripheral blood mononuclear cells (PBMC) (■), peripheral blood lymphocytes (PBL; PBMC depleted of monocytes) (□), T cells (●), T cells plus 5% autologous monocytes (○) and T cells plus 10% autologous B cells (▲) in the presence of $F(ab')_2$ fragments of autologous monoclonal IgG from one patient.

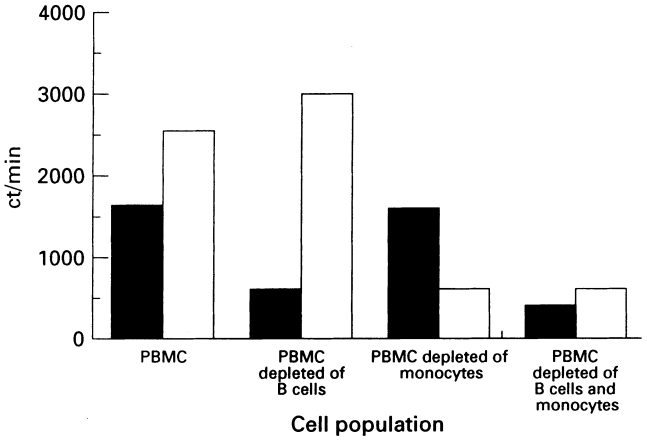

Fig. 4. Cell proliferation in the presence of low (1 ng/ml, ■) and high (10 μg/ml, □) concentrations of F(ab′)$_2$ fragments of autologous monoclonal IgG. Results from one patient are shown. PBMC, Peripheral blood mononuclear cells.

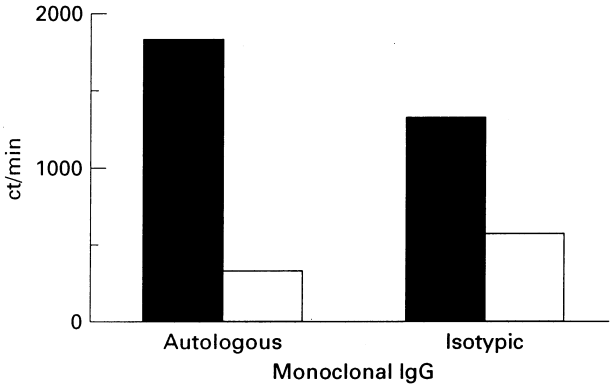

Fig. 6. Inhibition of anti-HLA-DR antibody on autologous or isotypic monoclonal IgG F(ab′)$_2$ fragment-induced cell proliferation after incubation of peripheral blood mononuclear cells (PBMC) in the absence (■) or presence (□) of 2 μg/ml of the mouse anti-DR MoAb from one patient.

monocytes was optimal for inducing T cell stimulation in response to PPD [17], we used 5% of monocytes in this study. As shown in Fig. 3, by adding monocytes back, cell stimulation induced by F(ab′)$_2$ fragments reappeared.

Interestingly, in the two patients whose cells showed a biphasic dose-dependent response, a difference in the cells required for antigen presentation was noted. As shown in Fig. 4, depletion of monocytes from PBMC reduced cell stimulation in response to the antigen at a high concentration, whereas the response to the antigen at a low concentration (1 ng/ml) was not affected. PBMC depleted of both monocytes and B cells ($CD14^+$ cells < 0·5%; $CD19^+$ cells < 0·1%) lost the ability to respond to the antigen at both the high and low concentrations. These results have been confirmed by using PBMC from the two patients analysed at two different times. The different requirement of cells for antigen presentation was further studied by using purified B cells or monocytes. As exemplified by the experiments in Fig. 3, cell stimulation induced by F(ab′)$_2$ fragments at high concentrations was dependent on monocytes, whereas that at low concentrations was B cell-dependent.

PPD-induced cell stimulation was abrogated by the depletion of monocytes from PBMC. Depletion of B cells had no effect. T cells alone did not respond to the antigen, while T cells plus 5–10% monocytes responded to PPD equally as well as PBMC (data not shown).

MHC dependence

An involvement of MHC class I/II molecules in cell stimulation was assessed by antibody blocking method. The effect of a mouse anti-HLA-DR MoAb on autologous F(ab′)$_2$ fragment-induced cell proliferation was shown in experiments exemplified by Figs 5–7. The anti-DR antibody, but not mouse isotypic normal IgG (0·02–2 μg/ml), induced a dose-dependent reduction in the proliferative response, whereas antibodies against HLA-DP, -DQ or HLA-ABC had no effect (data not shown). Such an MHC class II (DR)-restricted response was confirmed with cells from four patients. Cell stimulation induced by the autologous F(ab′)$_2$ fragments at both high and low concentrations was abrogated in the presence of 2 μg/ml anti-DR antibody (Fig. 5). Moreover, cell stimulation

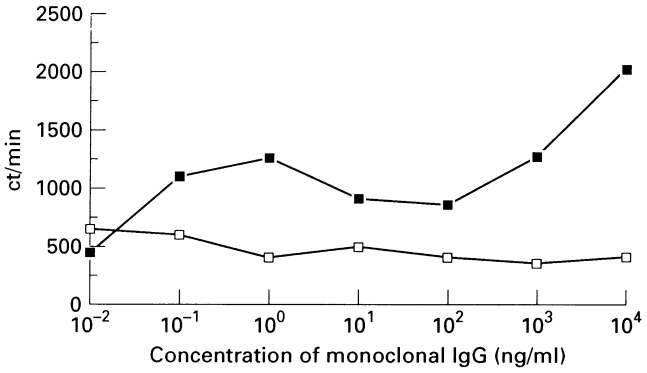

Fig. 5. Inhibition of anti-HLA-DR antibody on antigen-induced cell proliferation. Peripheral blood mononuclear cells (PBMC) were incubated in the presence of different concentrations of the autologous F(ab′)$_2$ fragments with (□) or without (■) the addition of 2 μg/ml of the mouse anti-DR MoAb from one patient.

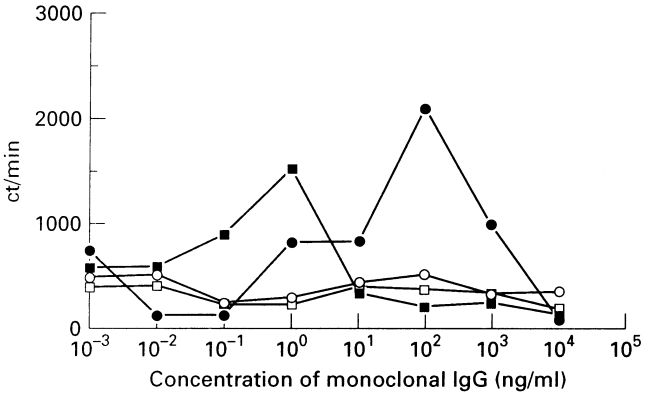

Fig. 7. Inhibition of anti-HLA-DR antibody on autologous monoclonal IgG F(ab′)$_2$ fragment-induced cell proliferation by pre- incubating it with purified B cells (□) or monocytes (○), before exposing them to T cells. Also shown is T cell stimulation induced by the antigen in the presence of B cells (■) or monocytes (●) without addition of the anti-DR antibody.

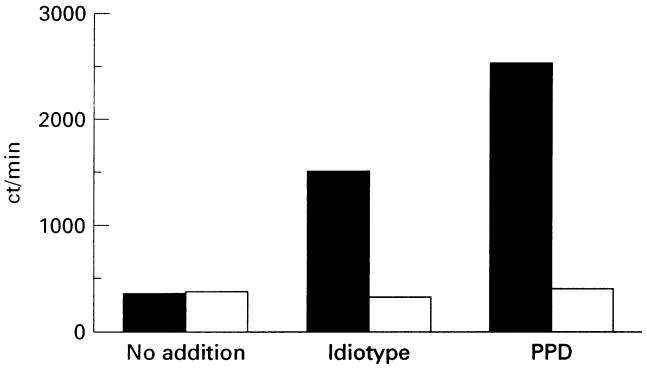

Fig. 8. Inhibition of chloroquine on antigen processing and presentation. Adherent cells were pulsed with idiotype (autologous IgG $F(ab')_2$ fragments) or purified protein derivative (PPD) in the presence (□) or absence (■) of 200 μM chloroquine. The cells were then washed and added to autologous T cells. Results from one patient are shown.

induced by isotypic $F(ab')_2$ fragments at a high concentration (10 μg/ml), which was noted in some patients, was also inhibited by the anti-DR antibody (Fig. 6). Comparable results were obtained in experiments in which purified monocytes or B cells were pre-incubated with the anti-DR MoAb or control IgG, before exposure to T cells (Fig. 7).

PPD-induced cell stimulation was also MHC-dependent. Antibody against HLA-DR resulted in 80% inhibition in cell proliferation, while antibody against HLA-ABC or mouse control IgG had no inhibitory effect (data not shown).

Effect of inhibitor

Cell stimulation induced by IgG $F(ab')_2$ fragments or PPD was totally abolished by the presence in the culture of chloroquine, while cell activation induced by Con A was not affected (data not shown). An inhibitory effect was also observed when the drug was present during the pulse of APC (adherent cells) with idiotype or PPD (Fig. 8).

DISCUSSION

Human T cells recognize conventional antigens as peptides in the context of MHC molecules [40], that are either class I (HLA-A, -B, and -C) or class II (HLA-DR, -DP, and -DQ) molecules. MHC class I molecules present mainly endogenously derived antigenic peptides and stimulate primarily $CD8^+$ T cytotoxic cells [41]. Exogenously derived peptides are preferably presented by MHC class II molecules and stimulate mainly $CD4^+$ T helper (Th) cells [42]. However, recent data obtained by studies of murine B lymphoma cells transfected with the gene encoding $\lambda 2$ light chain of MOPC315 [43,44], and through peptide elutions from MHC molecules [45], indicate that also endogenously derived peptides were presented by MHC class II molecules.

An important and unresolved question concerns how human T cells recognize idiotypic determinants on immunoglobulin molecules. Studies of Id-specific T cells from patients with multiple myeloma or from patients infected with *Schistosomiasis mansoni* showed that such T cells recognized conformational epitopes on Id^+ immunoglobulin molecules (direct binding) [10,12,16,25], and were not MHC-restricted [25]. To answer this question, we studied T cell responses to idiotypic structures on $F(ab')_2$ fragments of autologous monoclonal IgG from patients with monoclonal gammopathies, by measuring Id-induced cell proliferation and IFN-γ secretion. As a control, T cell stimulation induced by a conventional antigen PPD was also examined. The results indicate that T cell responses to idiotypic determinants required the presence of monocytes or B cells as APC, and that monocytes supported a high dose response (1–100 μg/ml), while B cells were required for a low dose response (10 pg/ml to 1 ng/ml). Antibodies against HLA-DR, but not -DP or -DQ or HLA-ABC, inhibited such T cell stimulation. Treatment of APC with a lysosomotropic agent (chloroquine) abolished cell activation. Thus, this study supports that $F(ab')_2$ fragments were processed by APC, and that peptide fragments containing idiotypic determinants were presented to $CD4^+$ Th cells in the context of class II (DR) molecules.

Generally, cells that express MHC class I and II molecules, such as monocytes or macrophages, dendritic cells and B cells, are competent APC (reviewed in [46]). However, a difference in the requirement of APC at different antigen concentrations is indicated in the present study. Depletion of monocytes from PBMC abrogated T cell stimulation induced by Id at high concentrations, while depletion of B cells abolished T cell stimulation induced by Id at low concentration. T cells alone and PBMC depleted of both monocytes and B cells did not respond to Id protein. This observation had been confirmed by testing T cells together with purified B cells or monocytes. These results indicate that monocytes presented Id peptides to T cells at high antigen concentrations, but only B cells, presumably the anti-idiotypic B cells that we have demonstrated in patients' peripheral blood [14,15], did so when Id protein was at very low concentrations. The plausible explanation is that, since monocytes do not express immune receptors for antigens, processing can presumably be initiated by pinocytosis of the antigen. Whereas B cells can bind antigen and their surface immunoglobulin serve to concentrate antigen for subsequent processing, they are far more effective APC, maximally stimulating T cells at antigen concentrations many-fold less than that required of non-specific APC such as monocytes or macrophages [47–49].

A biphasic dose response of IFN-γ-secreting cells and cell proliferation induced by $F(ab')_2$ fragments of the autologous monoclonal IgG was noted in the present and previous studies [16,17]. At low concentrations of monoclonal IgG (1 pg/ml to 1 ng/ml), autologous T lymphocytes from most patients responded, while allogeneic cells from other patients or healthy individuals were unstimulated. A 1000-fold higher IgG concentration resulted in a dose-dependent stimulation of both the autologous and allogeneic cells [17]. It was believed that the high-dose response to either the autologous or allogeneic (isotypic) monoclonal IgG was non-specific [16]. However, data from the present study demonstrated that such a response also required the presence of monocytes, and was inhibited by the anti-HLA-DR antibody. We believe that the high-dose response is also a specific T cell response, and that it may be a possibility that T cells with different specificities are involved.

Id-specific Th cells may regulate the production of both Id^+ immunoglobulin and anti-idiotypic antibodies against the Id^+ immunoglobulin. The present study indicates that Id-specific T cells recognized processed Id determinants in the context of MHC class II molecules. Such knowledge may help us to understand better the interactions between Id-specific Th cells and B cells, and provide a basis for functional studies with regard to the

regulatory role of Id-specific T cells on Id^+ B cell clone in monoclonal gammopathies.

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