Cell-mediated autoimmunity in patients with Wegener's granulomatosis (WG)

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SUMMARY
Despite the well described infiltration of cells of the cellular immune system in vasculitic lesions and the granuloma formation in patients with WG, the role of T cell-mediated autoimmunity in WG is not clear. Reports of T cell proliferation in response to neutrophil azurophilic granule proteins are contradictory. In this study we have assessed the proliferation of T cells of WG patients to purified proteinase 3 (PR3) and to total azurophilic granule proteins in two different assays. In addition to the classical proliferation assay with isolated peripheral blood mononuclear cells, we have used a whole blood proliferation assay. In both assays we found proliferative responses to PR3 in patients with WG. The number of patients reacting to the azurophilic granule extract was higher than the patients reacting to the purified PR3, suggesting that other autoantigens may also be involved. We have identified epitopes of PR3 that may be potential targets of class I-restricted T cell responses in the context of HLA-A*0201, the most common MHC class I molecule. These epitopes were determined by the binding of synthetic PR3 peptides to HLA-A*0201 on the antigen-processing defective cell line, T2. In addition, T cell lines were established from tissue biopsies, obtained from WG patients, and assessed for cytolytic reactivity against T2 cells, preloaded with synthetic PR3 peptides. We conclude that T lymphocytes of WG patients have increased proliferative responses to purified PR3 and to a larger extent to non-fractionated proteins of azurophilic granules of polymorphonuclear neutrophilic leucocytes (PMN).

Keywords proteinase 3 T cells vasculitis HLA-A*0201

INTRODUCTION
WG is characterized by a systemic necrotizing vasculitis of small and medium arteries in combination with crescentic necrotizing glomerulonephritis and development of granulomas in the upper airways, lungs and kidneys. The most frequent clinical manifestations are granulomatous lesions of the lower and upper respiratory tract and vasculitis in WG patients [3–6]. This model comprises intravascular activation of cytokine-primed neutrophils and/or macrophages by anti-neutrophil cytoplasmic antibodies (ANCA) followed by degranulation of the neutrophil and release of lysozymic enzymes and reactive oxygen radicals. ANCA are autoantibodies, found in the circulation of most WG patients, directed against several constituents of the azurophilic granules of neutrophils [7]. Based on the fluorescence pattern on ethanol-fixed granulocytes, ANCA can be divided in C-ANCA (cytoplasmic pattern), P-ANCA (perinuclear pattern) and aspecific ANCA. The most important target antigens are proteinase 3 (PR3) for C-ANCA and myeloperoxidase (MPO) for P-ANCA [7]. These antigens are expressed on the surface of cytokine-primed polymorphonuclear neutrophilic leucocytes (PMN) and are secreted upon cellular degranulation [8,9]. In addition to activating PMN, C-ANCA may interfere with the enzymatic activity of PR3 and with the inhibition of PR3 by α1 proteinase inhibitor [10]. Although levels of ANCA in the circulation tend to correlate with disease activity [7], not all aspects of the pathophysiology of WG can be attributed to the presence of ANCA. Massive cellular infiltrates in the renal interstitium, the nasal mucosa and (peri)vascular tissue of the lung, mainly consist of T cells, B cells and macrophages [11–14]. Extensive granuloma formation [14,15] suggests an important role of cell-mediated immune reactions in the pathogenesis of this disease. Furthermore, levels of soluble CD25 are elevated in patients with active WG, suggesting activation of the cellular immune system [16]. Moreover, a correlation was found between the number of T
lymphocytes in renal interstitium and renal function of patients with rapid progressive glomerulonephritis [12]. Lymphocyte proliferation induced by azurophilic granule proteins [13,17] is still controversial. In other autoimmune diseases, T cell responses against the target antigens of autoantibodies have been described, and have also been implicated in the pathogenesis of the disease, such as type 1 diabetes, autoimmune thyroiditis and myasthenia gravis [18–21].

We have investigated the cellular immune response to purified PR3 using the classical lymphocyte transformation test (LTT) and an adaptation of a whole-blood proliferation assay. This whole-blood assay requires only limited quantities of blood and no in vitro separation of blood cells [22]. Proliferative responses against exogenous antigens are generally MHC class II-restricted. However, since PR3 is also synthesized by endothelial cells [23–25], it may be presented in the context of MHC class I on endothelial cells and therefore be a target for autoreactive CD8⁺ T cells. Cytotoxic T cells have been implicated in the pathogenesis of several autoimmune diseases [26–28]. We have identified epitopes on PR3 that fit into the peptide-binding groove of HLA-A*0201 by determining binding of synthetic PR3 peptides to HLA-A*0201 on the processing-defective T2 cell line [29]. This strategy has been successful for determining cytotoxic T lymphocyte (CTL) epitopes on viral peptides and self-antigens that may act as targets for tumour-directed T cells [30–32]. We have attempted to use the potential epitopes of PR3 to detect PR3-specific HLA-A*0201-restricted T cells in T cell lines isolated from biopsies of inflamed tissues of WG patients.

**PATIENTS AND METHODS**

**Materials**

Azurophilic granules were prepared by subcellular fractionation of freshly isolated neutrophils [33] and the granules were extracted as described by Goldschmeding et al. [34]. PR3 was isolated from azurophilic granules as described in Leid et al. [35]. Both preparations were enzymatically active as determined by the cleavage of the synthetic substrate t-Boc-Ala-ONp (Sigma B5126). Enzymatic activity of PR3 and azurophilic granule extract was no longer detectable in the presence of 10% normal human serum.

The Q66–9 CTL clone specific for an influenza peptide was a kind gift of Dr H. Spits (Department of Immunology, Dutch Cancer Institute, Amsterdam, The Netherlands). Q66–9 is specific for the peptide GILGFVFTL of the influenza matrix protein in the context of HLA-A*0201.

The WG patients who were selected for this study received limited or no immunosuppressive therapy, or had a stimulation index (SI) higher than 2 in the corresponding proliferation assay upon stimulation with a recall antigen (tetanus toxoid (TT)). All patients were positive in the ANCA fluorescence assay, titres ≥ 64. Mean age of the patients was 52 years (range 28–87 years) and all patients had histology-proven WG. Healthy laboratory workers were used as controls.

The culture medium was Iscove’s modified Dulbecco’s medium supplemented with penicillin/streptomycin, glutamine, β-mercaptoethanol (IMDM). IMDM containing 10% pooled human serum (IMDM+) was used for most cell cultures.

**Lymphocyte transformation test**

Peripheral blood mononuclear cells (PBMC) were isolated from fresh heparinized blood by Ficoll amidotrizoate density separation. The cells were washed three times with PBS to remove platelets and washed once with IMDM+. The cells were resuspended in IMDM+ to a concentration of 1 × 10⁶ cells/ml and 100 µl were transferred to each well of 96-well U-bottomed plates (Costar, Badhoevedorp, The Netherlands). One hundred microlitres of PR3 (32 µg/ml), Lymphocult T (20%) or phytohaemagglutinin (PHA; 2 µg/ml) were added per well. The cells were incubated in a humidified incubator with 5% CO₂ at 37°C for 5 days, and 16 h before harvesting, 3H-thymidine was added (1 µCi/well; Amersham, Den Bosch, The Netherlands). Cells were harvested in a Skatron (Lier, Norway) cell harvester and 3H-thymidine incorporation was assessed using a LKB scintillation counter and Packard opti-fluor scintillation fluid. All experiments were performed in triplicate and background proliferation was usually less than 2000 cts/min with medium alone. Assays with higher background proliferation were not included in the results.

**Whole-blood proliferation assay**

This method was adapted from Bloemena et al. [22]. Heparinized peripheral blood of humans or rats was diluted 1:10 in IMDM and transferred in 150-µl volumes per well to a 96-well flat-bottomed plate (Greiner B.V., Alphen a/d Rijn, The Netherlands). Fifty microlitres of PR3 (4, 16 or 64 µg/ml) in IMDM were added per well and as a control 50 µl of Lymphocult T (40% in IMDM) or rat T cell growth factor (TCGF; 40% in IMDM) or IMDM alone were added. Incubation and harvesting were performed as described for the LTT. All experiments were performed in triplicate and background proliferation with medium alone was usually less than 200 cts/min.

**Immunization of the rats with PR3**

Five male Wistar rats (180–200 g) were immunized subcutaneously with 20 µg of PR3 in Freund’s complete adjuvant (FCA). After 14 days the rats were boosted with 20 µg of PR3 in Freund’s incomplete adjuvant (FIA). Venous blood (1-5 ml) was taken from the tail vein by incision 6 days after booster immunization. The blood samples were collected in sterile vials containing 75 U of heparin (Organon Technika, Boxtel, The Netherlands).

**Selection of synthetic peptides of PR3**

Nonamer, 10-mer and 11-mer sequences of PR3 were selected based on the consensus sequences of HLA-A*0201-binding peptides using two different computer programs. The consensus sequence used by the first program [30] is based on the sequence of peptides eluted from HLA-A*0201 [36,37]. The second program uses a consensus sequence based on the reported binding of synthetic peptides to HLA-A*0201 in vitro [48]. The peptides finally synthesized were those selected from the list of peptides generated by both programs. This selection was based on the rankings of the peptides in the list and on the hydrophobicity of the peptide. Peptides were synthesized using Fmoc chemistry on an Ahmed AMS 422 automated multiple peptide synthesizer. Purity of peptides was more than 90% as determined by reverse-phase high performance liquid chromatography (HPLC) analysis. Extremely hydrophobic peptides were excluded due to their insolubility. A list of the 28 synthetic peptides synthesized and tested for in vitro binding to
The maximal fluorescence index (FI) induced by the peptide is indicated in the fourth column (— indicates FI < 0). A + in the last column indicates that at a concentration of 25 μg/ml or lower an FI of at least 1 was induced by the peptide. The (putative) anchor residues are underlined.

The maximal FI induced by a peptide and the maximal dilution of a peptide resulting in an FI higher than 1 were used as criteria for the binding of a peptide (Table 1). Maximal FI was usually found at 100 μg/ml of peptide, although for some peptides the FI was maximal at 50 μg/ml.

### T cell lines from biopsies

Needle biopsies from kidneys and biopsies from lung, skin, nasal mucosa or salivary gland were cut into very small pieces and transferred to 24-well culture plates containing 1 ml of IMDM + 10% Lymphocult T (LcT; Biotest). After 10–14 days of culture the proliferating T cells were restimulated with irradiated pooled human lymphocytes and 1 μg/ml of PHA in IMDM + 10% LcT. After 12 days of culture portions of the T cells were frozen in liquid nitrogen. The rest of the T cells were restimulated as described and tested in a cytotoxicity assay 7 days after restimulation.

### Cytotoxicity assay

T2 cells (2 × 10⁶) or Epstein–Barr virus (EBV)-transformed B cells were labelled with 150 μCi of sodium ⁵¹chromate for 1 h at 37°C, washed twice with IMDM and resuspended in IMDM + at a concentration of 4 × 10⁶ cells/ml. Sufficient numbers of labelled cells were incubated with 20 μg/ml (final concentration) of peptide for 15 min at 37°C or without peptide and then transferred to 96-well plates (2000 cells/well). Various numbers of T cells were added to the wells, resulting in effector:target ratios of 100, 50, 25 and 12.5 and the plates were centrifuged for 5 min at 1400 rev/min. As controls 50 μl of IMDM + (spontaneous release) or 2% Triton X-100 (maximal release) were added instead of T cells. Supernatant (50 μl) was harvested after 4 h of incubation at 37°C and counted in a Packard gamma counter. The percentage of ⁵¹Cr release was calculated as follows:

\[
\text{ct/min experimental well} - \text{ct/min spontaneous release} \times 100\%
\]

### Statistical analysis

Student’s t-test for paired data was used to calculate P values of differences between rats before and after immunization. The upper limit of the normal controls was calculated as follows: mean SI ± 2 × s.d. Using this limit, P values were calculated by \( \chi^2 \) Fisher’s exact test.

### RESULTS

To find out whether circulating PR3-specific T cells were present in WG patients we tested PBMC of 21 patients and eight healthy controls in an LTT for proliferative responses against PR3. In the patient group the SI of five patients exceeded the upper limit of the controls (mean SI ± 2 × s.d. = 2.48) and therefore should be considered positive (Fig. 1). The mean SI of the total patient group in response to 16 μg/ml PR3 was slightly higher than the mean SI of the controls (mean ± s.d. 1.66 ± 0.90 and 1.14 ± 0.67, respectively), but these differences were not statistically significant (P = 0.16). Lower concentrations of PR3 resulted in lower responses in those patients who responded to PR3. No significant difference in proliferation was found between patients with active disease (●) or with non-active disease (○).
Cell-mediated autoimmunity in WG patients

Patients

Controls

Fig. 1. Proliferation of lymphocytes from patients and controls against 16 μg/ml proteinase 3 (PR3) in a lymphocyte transformation test (LTT). Proliferation is indicated as stimulation index (SI; ct/min with PR3/ct/min with medium alone). The upper limit of the controls (mean ± 2 x s.d.) is shown as a dashed line. ●, Patients with active disease.

To test a larger group of patients, an alternative whole-blood proliferation assay requiring only limited amounts of blood was used. This assay utilizes non-fractionated peripheral blood diluted in culture medium, without addition of serum proteins. First, to test the assay conditions, we set up an animal model for the T cell responses to PR3 in rats. We performed whole-blood assays comparable to the human assay with PR3-immunized Wistar rats (n = 5). All rats responded to PR3 (Fig. 2a) and a significant proliferation against PR3 was found with 1, 4 and 16 μg/ml PR3 (mean ct/min ± s.d. medium, 109 ± 10; PR3, 2190 ± 625 and 3892 ± 1054, respectively, P < 0.05 (paired t-test) for each concentration of PR3). Some rats responded even to 0.05 μg/ml of PR3 (data not shown). PR3 seemed to have a slight mitogenic effect at 16 μg/ml in non-immunized rats, but this was statistically not significant (ct/min of medium 120 ± 18; PR3 16 μg/ml, 152 ± 3 (mean ± s.e.m.; P = 0.129). Sera of all rats diluted up to 1:640 were positive in an ELISA using purified PR3 (Fig. 2b).

Having shown the potential use of this assay in rats, we tested 36 patients and 11 healthy controls in whole-blood proliferation assays (Fig. 3). Cellular proliferation against 16 μg/ml PR3 and 80 μg/ml of a non-fractionated extract of azurophilic granules (granule extract) was tested. Four patients out of 36 had an SI against PR3 exceeding the upper limit of the controls (mean ± 2 x s.d. = 1.42 ± 2 x 0.58 = 2.58) and were considered positive. For the whole patient group, differences in SI between patients and controls were not significant (P = 0.33).

An almost significant difference between patients and controls was found for granule extract-induced proliferation (1.19 ± 0.90 and 0.77 ± 0.26, respectively (mean ± s.d.), upper limit of controls = 1.29; P = 0.063). Fourteen patients had an SI against the granule extract exceeding the upper limit of the controls. Lower concentrations of antigen generally resulted in lower responses of those patients who responded to PR3 or the azurophilic granule extract. No significant correlation could be found between the reaction against PR3 and against the granule extract. However, three of the four patients with positive response against PR3 also had a positive response against the granule extract. No significant differences in proliferation between patients with active disease (filled symbols) and non-active disease (open symbols) were found. Correlations between the responses to PR3 in the LTT and in the whole-blood assay could not be calculated since patient groups of both assays were not related and only a few patients were tested in both assays at the same time.

Since the 95% CI of the mean of the controls (1.03–1.81,
mean = 1.42) does not equal 1, it can be concluded that PR3 had a slight but significant mitogenic effect (P ≤ 0.05). Similarly, it can be concluded that azurophilic granule extract had a slight inhibitory effect on the proliferation of control lymphocytes (mean SI = 0.77, 95% CI of the mean = 0.61–0.94) (P ≤ 0.05). In this assay, an SI in response to TT of almost 50 could be found in some patients, indicating the efficiency of the assay. No correlation was found between the responses against TT and PR3 or granule extract (data not shown).

Since PR3 is synthesized by endothelial cells after stimulation with tumour necrosis factor-alpha (TNF-α) [24], we investigated whether cytotoxic T cell responses to PR3 in the context of class I MHC were detectable in T cell lines of WG patients.

We selected and synthesized 28 synthetic peptides of 9–11 amino acids (Table 1), based on two computer models predicting the binding of peptides to HLA-A*0201 [30], unpublished results). Actual binding of these peptides to HLA-A*0201 was tested using the processing-defective T2 cell line. Peptides 3, 4, 13, 18 and 23 induced at least two-fold up-regulation of the mean fluorescence at concentrations of 25 μg/ml and higher (FI ≥ 1). Therefore these peptides are considered to be effectively binding to HLA-A*0201 (Fig. 4). Peptides 9, 11, 12 and 16 also induced a maximal FI higher than 1.0 at concentrations of 50–100 μg/ml. This up-regulation was no longer detectable at concentrations of 25 μg/ml and lower, and these peptides were therefore not used in the cytotoxicity assays (Table 1).

Peptides 3, 4, 13, 18 and 23 were used to study the cytotoxic potential of biopsy-derived T cells of HLA-A*0201-positive WG patients. HLA-A2 frequencies are not different in WG patients compared with the normal population [38–40]. HLA-A*0201 is the major subtype of A2 and is found at high frequencies in all human races [41]. The presence of HLA-A*0201 on patient lymphocytes was detected with a cytotoxicity assay using EBV-immortalized B cells of HLA-A2-positive WG patients. After incubation of the B cells with a HLA-A*0201-binding peptide of the influenza matrix protein, these cells served as targets for the influenza-specific HLA-A*0201-restricted CTL clone Q66-9 [31]. Of the five A2-positive B cell lines tested, four were HLA-A*0201-positive and were efficiently lysed by the Q66-9 CTL clone after incubation with the influenza peptide (data not shown).

Four T cell lines were obtained from biopsies of HLA-A*0201-positive patients taken at sites of inflammation (nasal mucosa, lung, salivary gland and skin). Each cell line was tested for cytotoxic capacity against 51Cr-labelled T2 cells preincubated with each of the five selected peptides or medium. None of the peptides induced cytotoxicity higher than the background lysis of T2 cells preincubated with medium alone. All T cell lines contained both CD4+ and CD8+ T cells. A representative experiment is shown (Fig. 5).

**DISCUSSION**

T cell-mediated autoimmune reactions have been suggested as
an important factor in the pathogenesis of WG and other
vasculitides, not only because of the influx of T lymphocytes
in inflamed tissues, but also because of the formation of
granulomas in WG patients [14,22]. Secondary evidence for
T cell-mediated inflammatory processes in WG is found in the
enhanced levels of sCD25 in the circulation of patients with
active disease [16]. Although contradictory descriptions of the
proliferative responses of PBMC of WG patients to PR3 and
other constituents of the azurophilic granules of neutrophils
have been published [17,42], the positive results suggest that T
cell reactivity against target antigens of ANCA could be
important in WG. Furthermore, the predominance of IgG4–
C-ANCA in sera of WG patients suggests repeated stimulation
of PR3-specific B cells by (IL-4-producing) PR3-specific, T
helper cells [43,44].

In the present study, we tested the proliferative capacity of
T cells of WG patients against PR3 and an extract of azuro-
philic granules from PMN. In a classical proliferation assay
with isolated PBMC we observed that five patients from a panel
of 20 specifically proliferated in the presence of PR3. To test a
larger group of patients we used a modified proliferation assay
that used whole blood instead of isolated PBMC. Using PR3-
immunized rats, it was found that the optimal PR3 concentra-
tion (16 \mu g/ml) induced strong proliferative responses in whole
blood of immunized rats without mitogenic effects in non-
immunized animals. Similar results were found with TT in
TT-immunized rats (van Breda-Vriesman, unpublished data).
Using the optimal PR3 concentration we tested a larger group
of patients for PR3-specific proliferation in the whole blood
assay. As in the LTT, a limited group of patients had a
significant proliferative response to 16 \mu g/ml PR3. Surpris-
ingly, a larger percentage of the patients tested (14/36), reacted
to a non-fractionated azurophilic granule extract. This finding
suggests that other constituents of azurophilic granules may be
targets of autoimmune responses. These antigens may include
myeloperoxidase, cathepsin G and elastase, all of which have
been described as target antigens for ANCA [7]. Our findings
confirm the proliferative response of lymphocytes from WG
patients to azurophilic granule extract, as shown by Rasmussen
& Petersen [13]. Other investigators have not found specific
proliferation of patient lymphocytes using a total cytoplasmic
extract of neutrophils [17]. This latter finding may reflect the
method of antigen preparation (acid extraction). Another
possible explanation for the higher response to azurophilic
granule extract may be found in the observation that auto-
reactive CD4+ T cell clones from lupus-prone mice specifically
respond to nucleosomes, complexes of DNA and histones,
while purified histones or DNA alone do not stimulate these
clones [45]. The authors suggest that, during processing and
presentation of these complexes, certain epitopes are protected
against proteolysis, whereas these epitopes are degraded during
processing of the purified histones. Similarly, PR3 may be
present in azurophilic granules in the form of multimers or
complexes with other molecules. During isolation of PR3 these
complexes may be disrupted by the Triton X-100 used for the
extraction of the granules, while the non-fractionated azuro-
philic granule extract used in the proliferation assays is not
treated with detergent.

Another protein that can be a potential target for T cell-
mediated autoimmune responses is \alpha-enolase, a neutrophil
cytosolic protein related to heat shock proteins. Antibodies
against this enzyme have been described in the sera of both WG
and systemic lupus erythematosus (SLE) patients with renal
involvement [46]. T cell responses to heat shock proteins have
been described in various autoimmune diseases, such as rheu-
matoid arthritis [47].

Since PR3 is synthesized by cytokine-activated endothelial
cells, PR3 peptides may be presented in the context of MHC
class I and thus be a target for autoreactive cytotoxic T cells.
We have identified several peptides of PR3 that may be
presented in the context of the common MHC class I molecule
HLA-A*0201. The programs used to select the peptides to be
synthesized and tested for binding to HLA-A*0201 were either
based on the sequences found in peptides eluted from HLA-
A*0201 [31,36] or derived from the consensus sequence of an
extended set of synthetic peptides known to bind to HLA-
A*0201 in vitro [48]. Peptides 4, 13 and 18 all fulfilled the motif
defined by Rammensee [31] (leucine or isoleucine at position 2
and valine or isoleucine at position 9 or 10), while peptide 4 was
strongly selected by both programs. Peptide 3 has the correct
anchor residues, but the other residues do not match with the
residues found in naturally processed peptides, and therefore
peptide 3 was not selected by the first program. Peptide 23
(FVLTAAHCL) has valine as anchor on position 2, which is
not found in naturally processed peptides on HLA-A*0201
[36]. However, valine at position 2 is incorporated in the
consensus sequence used by the second program, as it supports
in vitro binding of synthetic peptides to HLA-A*0201. Although
it is not clear whether such peptides are processed naturally,
synthetic peptides of the human papillomavirus type
16 (HPV-16) oncogenes E6-E7, selected by binding to class I,
can be effective for the induction of peptide-specific CTL, that
lyse tumour cells in vitro [49]. Attempts to detect CTL activity
against the HLA-A*0201-binding PR3 peptides in T cell lines
derived from biopsies of WG patients were uniformly unsuc-
cessful. This failure might be due to the low frequencies of
peptide-specific CTL in the biopsy tissue or to the culture
conditions that do not favour outgrowth of CTL. It may be
necessary to add IL-12, a potent stimulator of the cellular
immune response [50], to generate T cell lines with higher
frequencies of PR3-specific CTL. Attempts to generate PR3
peptide-specific T cell clones out of biopsy T cell lines, using
peptide-loaded autologous EBV-transformed B cells as stimu-
lator cells, were not successful either. Generation of peptide-
specific T cell lines from peripheral blood of WG patients
by stimulation with peptide-loaded T2 cells (as described
previously [31]) may provide a different way to detect the presence
of PR3-specific CTL.

Not much is known about the activation of autoreactive
T cells in autoimmunity. In chronic active hepatitis it was
found that liver-derived T cell clones of both the CD4 and
dCD8 subset are stimulated by class II or class I matched
hepatocytes, respectively, but not by autologous PBMC
[51]. This suggests that autoantigens produced by hepatocytes
are presented not only in the context of class I but also
in the context of class II. This presentation is confirmed by
studies that show that cytosolic proteins can be presented in
the context of class II [52]. Since PR3 is synthesized by
human endothelial cells and murine endothelial cells are
able of processing and presenting exogenous antigens to T
cells in the context of class II after stimulation with interferon-
gamma (IFN-\gamma) [53], it seems possible that CD4+ and CD8+
PR3-specific T cells could be activated by vascular endothelial cells.

Endothelial cells presenting PR3 peptides in the context of class I or class II molecules may also be a direct target of cytotoxic activity of either CD8+ or CD4+ autoreactive T cells. It has been described that hepatocyte-specific T cells of both the CD4 and the CD8 phenotype possess cytotoxic capacity [51]. Cytotoxic autoreactive T cells have been described in type I diabetes mellitus [54] and CTL specific for autologous biliary epithelial cells have been described in primary biliary cirrhosis patients [28].

In conclusion, we have demonstrated that PR3-specific T cells are present in the circulation of some WG patients. Furthermore, the proliferation to azurophilic granule extract peptides suggests that T cell recognition of other azurophilic granule proteins occurs. T cell lines and clones against PR3 or other granule antigens are needed for further analysis of both the cytokine pattern and fine specificity of autoreactive T cells in WG. Furthermore, PR3 induces strong proliferative responses in whole blood assays using blood of PR3-immunized rats. We conclude that this whole blood assay is suitable for the longitudinal study of cellular (auto)immune responses in laboratory animals, and it may therefore serve as a potent tool for the study of animal models of vasculitis or glomerulonephritis.

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REFERENCES

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41 The databook of the 11th International Histocompatibility Workshop (Yokohama, Japan) 1991; 2:807.