Association of a syndrome resembling Wegener’s granulomatosis with low surface expression of HLA class-I molecules

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Summary

Background Granulomatous syndromes, such as Wegener’s granulomatosis, are defined according to complex criteria, but the underlying cause is rarely identified. We present evidence for a new aetiology for chronic granulomatous lesions associated with a recessive genetic defect, which is linked to the human leucocyte antigen (HLA) locus.

Methods Five adults with necrotising granulomatous lesions in the upper respiratory tract and skin, associated with recurrent bacterial respiratory infections and skin vasculitis, were identified. A diagnosis of Wegener’s granulomatosis was considered in all of them, but abandoned because of an incompatible disease course and resistance to immunosuppressive treatments. Peripheral blood samples were taken and analysed by immunohistochemistry and fluorescent-activated-cell-sorter analysis. Since all five patients were homozygous for the HLA locus, we looked for genetic defects located within the HLA locus with PCR and restriction fragment length polymorphism.

Findings A severe decrease in cell-surface expression of HLA class-I molecule was seen in all patients. Defective expression of the transporter associated with antigen presentation (TAP) genes was responsible for the HLA class-I down-regulation, and in two patients we identified a mutation in the TAP2 gene responsible for the defective expression of the TAP complex. We showed the presence of autoreactive natural killer (NK) cells and γδT lymphocytes in the peripheral blood cells of two patients. Correction of the genetic defect in vitro restored normal expression of HLA class-I molecules and prevented self-reactivity in the patients’ cells. Histology of granulomatous lesions showed the presence of a large proportion of activated NK cells.

Interpretation Our findings define the cause and pathogenesis of a new syndrome that affects patients with a defective surface expression of HLA class-I molecules. The syndrome resembles Wegener’s granulomatosis both clinically and histologically. Patients have chronic necrotising granulomatous lesions in the upper respiratory tract and skin, recurrent infections of the respiratory tract, and skin vasculitis. A predominant NK population within the granulomatous lesions suggests that the pathophysiology of the skin lesions may relate to the inability of HLA class-I molecules to turn off NK cell responses. Accurate genetic analysis of a defined syndrome can provide a better understanding of the cause and pathogenesis of a disease.

Lancet 1999; 354: 1598-603

Introduction

Patients with systemic inflammatory disease present the clinician with a diagnostic challenge, because the causes are unclear in many cases and reliable diagnostic tests are not available. Diagnosis in individual patients is generally based on international classification criteria, which include clinical, histological, and serological measurements. We describe five patients with a syndrome of chronic necrotising granulomatous lesions, small-vessel vasculitis, recurrent respiratory-tract infections, and development of bronchiectasis. A diagnosis of Wegener’s granulomatosis was considered in all five patients during the disease course. The American College of Rheumatology 1990 criteria and the Chapel Hill 1992 consensus definition could be applied to classify the disease as Wegener’s granulomatosis in three of five patients. We report the investigation and subsequent classification of these patients.

Methods

The index patient (patient 1) was clinically characterised at the Rheumaklinik Bad Bramstedt in 1996. This patient’s striking clinical features, and the fact that her first-degree relative (patient 3) had similar skin lesions, prompted us to look for patients with similar clinical features. We identified other patients by searching the Medline database and contacting several dermatology departments. All patients described in this paper are female.

Antibodies specific for CD94 (HP3B1; IgG), and immunoglobulin-like transcript 2 (ILT 2; 3F1) were used for the immunohistochemistry and fluorescence-activated-cell-sorter analysis. γδT cells were stained with the monoclonal antibody B1 (IgG1). The variable α1-chain (Vα1)-specific antibody A13, and variable α2-chain (Vα2)-specific antibody, 4G6 (IgG1) (provided by G De Libero, Kantonsspital Basel, Switzerland), were used to measure the α1 to α2 ratio and for immunohistochemistry.

2×10⁶ B cells of each patient were infected for 1 h at 37°C with the recombinant vaccinia virus encoding the human TAP1 and TAP2 proteins (TAP VAC) (provided by J Yewdell, Bethesda, USA) at 5 plaque-forming units per cell and stained
HLA haplotype, HLA class-I expression, and lymphocyte subpopulation from peripheral blood

with W6/32 and a fluorescein-isothiocyanate-labelled antibody to mouse IgG6, before fluorescence-activated-cell-sorter analysis. The TAP2 coding sequence was determined on both strands by DNA sequencing with an Applied Biosystems automated sequencer (Perkin-Elmer, Warrington, UK). PCR of exons 5 and 6 of the TAP2 gene was done with the forward primer 5'-TGGTGTTTGCTGGCCCTC-3' (position 4581) and the reverse primer 5'-GAGAGCAGGCTTGGCTTC-3' (position 46354). Analysis by restriction-fragment length polymorphism was done with the HpaII restriction enzyme.

γδT cells derived from patients 2 and 3 were sorted with the pan γδ antibody B1 and cloned in 96-well plates at 1–5 cells per well.γδ clones were maintained in Iscove medium, containing 5% human serum plus 200 U recombinant interleukin 2. Irradiated B cells (LG2) and peripheral-blood mononuclear cells from normal volunteers were used as feeder cells. Natural killer (NK) cell lines from patient 2 were obtained after two rounds of negative selection with antibodies to CD3 and CD14 and restimulated in Iscove medium containing 5% human serum plus 1000 U recombinant interleukin 2. The B cells LG2 and B cells from patient 2 were irradiated and used as feeder cells for natural killer cells. Frozen sections were stained by streptavidin-biotin immunoperoxidase technique and counterstained with M ayer haematoxylin. Metabolically labelled HLA class-I molecules were immunoprecipitated with W6/32 from patient 1 or from the positive control B-cell line. Cells were lysed either immediately after sulphur-35-labelled methionine pulse or after 3 h or 15 h.

Tissue typing of all five patients showed that they were homozygous for their HLA haplotype, and fluorescence-activated-cell-sorter analysis of patients' peripheral-blood mononuclear cells showed very low surface expression of HLA class-I molecules compared with positive control samples (table). However, the level of expression of HLA class-I molecules was lower than that in controls (data not shown). Down-regulation of HLA class-I molecules was controlled by a recessive gene, since the parents of patient 1 were healthy and showed normal expression of HLA class-I molecules (data not shown). This finding suggested that the recessive genetic defect leading to the low HLA class-I expression maps to the HLA locus. The finding that HLA haplotypes were not shared by any patients, with the exception of the related patients, suggests that class-I down-regulation was not linked to specific HLA class-I alleles. However, the HLA

<table>
<thead>
<tr>
<th>Patient</th>
<th>HLA haplotype</th>
<th>Defect</th>
<th>CD3+ (% of total PBMC)</th>
<th>CD3- (% of total PBMC)</th>
<th>All γδ cells</th>
<th>V61</th>
<th>V62</th>
<th>HLA class I</th>
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<tr>
<td>1</td>
<td>A<em>11B</em>1502Cw<em>0801DRB1</em>1505QB1*0601</td>
<td>TAP2 mutation</td>
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<td>19</td>
<td>8</td>
<td>32</td>
<td>32</td>
<td>23</td>
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<tr>
<td>2</td>
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<td>TAP2 mutation</td>
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<td>11</td>
<td>4</td>
<td>ND</td>
<td>14</td>
<td>17</td>
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<td>3</td>
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<td>TAP2 mutation</td>
<td>39</td>
<td>24</td>
<td>8</td>
<td>16</td>
<td>14</td>
<td>12</td>
</tr>
<tr>
<td>4</td>
<td>A<em>11B</em>1502Cw<em>0801DRB1</em>1505QB1*0601</td>
<td>TAP2 mutation</td>
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<td>88</td>
<td>0-2</td>
<td>6</td>
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<td>0-5</td>
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<td>6</td>
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<td>27</td>
<td>8</td>
<td>5</td>
<td>ND</td>
<td>4-5</td>
</tr>
</tbody>
</table>

N D = not done; PBMC = peripheral-blood mononuclear cells.
haplotype of patient 2 is identical to that of a previously described HLA class-I-deficient male patient with a TAP1 mutation, who had bacterial respiratory infections and vasculitis.8,9

Despite the severely reduced HLA class-I surface expression, significant numbers of cells positive for CD3 and CD8 were detected in the peripheral-blood mononuclear cells of all patients (table). The proportion of lymphocyte subpopulations varied substantially between patients. In particular, an inversion of the ratio of CD4/CD8 cells was noted in patient 4, who had very low numbers of cells positive for CD3 and CD56 compared with the other patients. Exposure to pathogens could have altered the CD4/CD8 ratio in this patient and skewed her T-cell repertoire. A substantial expansion of (CD3-negative, CD56-positive) NK cells and \( \gamma \delta \) T cells was recorded in samples from patient 1 and, to a lesser extent in patients 2, 3, and 5, whereas cells positive for CD3 and CD56 were increased in patient 5 (table). The proportion of NK cells in patient 1 fluctuated over 3 years (7–32%), therefore the values of patients' NK cells, \( \gamma \delta \) T cells, CD56, and CD57 in the table may have been affected by environmental factors such as recent infections and treatments at the time blood samples were analysed.

Figure 1: Ulcerating skin lesions on midface and legs

Staining of patients' peripheral-blood mononuclear cells and \( \gamma \delta \) bulk cultures with V\( \delta 1 \)-specific and V\( \delta 2 \)-specific antibodies showed that the proportion of \( \gamma \delta \) T cells with the V\( \delta 1 \)-T-cell receptor, rather than the V\( \delta 2 \)-T-cell receptor, was greater in the patients' samples than in control peripheral-blood mononuclear cells (table). The stability and maturation of HLA class-I molecules was analysed in patients 1–4. Pulse-chase analysis of HLA class-I molecules from the B cell lines of patient 1 showed that newly synthesised HLA class-I molecules were associated with \( \beta \_2 \)-microglobulin, but did not mature in the endoplasmic reticulum after 15 h chase. Moreover, transport of a radiolabelled peptide into the endoplasmic reticulum of patient 1 was completely abolished, clearly implicating a defect of the TAP complex. Infection of the B cells of patient 1 with a recombinant vaccinia virus encoding the full length Epstein-Barr viral LMP2A protein did not sensitise them for lysis by LMP2A-specific A11-restricted cytotoxic T lymphocytes, whereas presentation of the same epitope, as a synthetic peptide, was not affected (data not shown). Western blotting of the B-cell lysate of patient 1 showed normal expression of TAP1 (data not shown), whereas TAP2 was not detected by antibodies specific to carboxy terminal residues of the TAP2A and TAP2B proteins (figure 2).

Figure 2: Expression of TAP1/TAP2 complex in patients 1 and 2

Lane T2: B-cell line bearing a deletion of the TAP-1 and TAP-2 genes (negative control).
HLA class-I expression was caused by a defective TAP complex, B cells from patient 1 were infected with a recombinant vaccinia virus encoding the human TAP1/TAP2 proteins, which restored normal surface expression of class-I molecules (figure 3).

Expression of the TAP complex was analysed in B cells from patient 2 (figure 2) patients 4 and 5 (table). TAP1 protein was not detectable by western blot in these B-cell lines. However, a very faint trace of a TAP2 protein was detected in the lysates of these patients’ B cells. This finding is consistent with the possibility that the lack of TAP1 protein may reduce the steady-state amount of the TAP2 protein. Infection of B cells from patients 2 (figure 3), 4, and 5, and the fibroblasts of patient 5 with TAP vaccinia, upregulated surface expression of HLA class-I molecules.

We then sought to address whether in patients with a germline down-regulation of HLA class-I molecules, the activity of cells expressing HLA class-I binding inhibitory receptors was altered. Several γδ-T-cell clones were generated from patients 2, 3, and 4 and their phenotype and activity analysed. All γδ-T-cell clones from patient 3, rapidly proliferating from the γδ T-cell bulk culture, were V1V9 and they all expressed the HLA class-I binding receptors ILT2 and CD94/NKG2A (data not shown). Cytotoxic activity of three γδ clones from patient 3 was tested against the class-I-negative cell line 721.221, the HLA-identical TAP2-negative B cells from patient 1 and, as control, against class-I-positive B cells (figure 4A and B). We showed that 721.221 cells were lysed by the three γδ T-cell clones. Whereas a higher percentage of cytotoxicity was observed against B cells of patient 1 than against 721.221 clones. These results were confirmed with autologous B cells from patient 3 (data not shown). We found that lysis of target cells was abolished after the level of surface expression of HLA class-I molecules was

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**Figure 3:** Expression of wild-type TAP proteins in patients 1 and 2 B cells restores HLA class-I surface expression

Cell lines were stained for surface expression of HLA class I after infection overnight with a vaccinia virus encoding the human TAP1 and TAP2 proteins (TV), or a vaccinia virus encoding the influenza matrix protein (V), as a negative control. Staining with the secondary antibody alone is shown as a dotted line.

**Figure 4:** Activity of NK and γδ T cells

A and B: Three γδ T-cell clones (1, 7, and 12) from patient 3 were tested against the HLA-identical TAP-2-negative B cells from patient 1 as target cells. In the experiment done with clone 7, target cells were infected overnight with TAP-vaccinia (TV) or as a control with an irrelevant vaccinia virus (V).

C: The γδ clone 6G11 from patient 2 was tested against autologous B cells as target cells. Targets were infected with irrelevant vaccinia (V) or with the TAP-vaccinia (TV).

D: An NK cell line from patient 2 was tested against autologous B cells and B cells from patient 1 as target cells. The class-I-negative B-cell line 721.221 K562 cells, and class-I-positive B cells lines (BCL) were used as controls. Each bar corresponds to a different effector-to-target ratio.
increased, after infection with TAP vaccinia (figure 4B). This finding suggests that γδ-T-cell inhibitory receptors were capable of transducing inhibitory signals. We found that four NK clones from patient 3 were not autoreactive, although they were capable of killing K 562 cells (data not shown). Analysis of NK cells and γδ T cells was also done for patient 2. We confirmed that several Vβ1 positive γδ clones were autoreactive, and that lysis of autologous cells was abolished after the clones expressed wild-type TAP1 and TAP2 proteins, and restored normal surface expression of HLA class-I molecules (figure 4C). Similar experiments were then done with a polyclonal NK cell line. We showed that NK cells from patient 2 were autoreactive, as defined by their ability of lysing autologous B cells (figure 4D).

The presence of autoreactive NK cells and γδ T cells in peripheral-blood mononuclear cells suggests that these cells may be involved in the pathogenesis of necrotising skin lesions. Biopsy samples were collected from skin lesions of patient 2, and their histology showed the presence of several foci of cells infiltrating the dermis. To define the phenotype and origin of infiltrating cells, sections were stained with a large panel of different monoclonal antibodies. The results of this analysis (figure 5) showed that a large proportion of infiltrating cells were NK (CD3-CD56+), which expressed high levels of the inhibitory receptors CD94 and ILT2, and were HLA class-II positive. A small but significant proportion of cells were γδ CD3 positive.

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Discussion

The patients had a striking homogeneity of clinical symptoms and disease course. These clinical features might be associated with a recessive genetic defect, which maps to the HLA locus. In a previous report, similar respiratory symptoms including bronchiectasis were described in TAP2-deficient children, but chronic granulomatous inflammation in the upper respiratory tract and the skin were not reported. Chronic inflammatory lesions in HLA class-I-negative patients might require exposure to pathogens or to other triggering events. Previous single case reports noted sinobronchial disease and skin lesions on the legs of patients with low HLA class-I expression; skin lesions were described as ulcers or as plaques resembling necrobiosis lipoidica. HLA class-I deficiency in these patients has been shown to be caused by TAP1 mutations.

The pathogenesis of such chronic inflammatory lesions is still not clear. The presence of few multinucleated giant cells in the granulomatous lesions suggests that tissue damage might have been caused by mycobacterial infection. However, in all patients tuberculostatic treatments did not give any longlasting clinical benefit, and mycobacteria were not identified in the lesions by tissue stainings and culture techniques. An alternative possibility is that the chronic inflammation was caused by autoreactive lymphoid cells, such as the expanded populations of NK cells or γδ T cells found in these patients. This hypothesis rests on the finding that the self-reactivity of these cells is negatively regulated by HLA class-I-binding receptors, such as NK-cell inhibitory receptors and the recently characterised CD94/NKG2 receptor. We showed the presence of self-reactive γδ T cells in patient 3 (figure 4A and B), and in patient 2 we showed a massive infiltration of NK cells within the granulomatous lesions associated with self-reactive NK cells and γδ T cells in the patient’s peripheral-blood mononuclear cells (figure 4C and D, figure 5). Progression of the granulomatous lesions in patients 1 and 4 after treatment with interferon alfa is of interest, because activation and cytotoxicity of NK cells is enhanced by interferon alfa. These findings strongly suggest that in patients with a germline down-regulation of HLA class-I molecules, the threshold of inhibition provided by killing inhibitory receptors may not be reached, leading to autoimmune lesions. Self-reactive NK cells and γδ T lymphocytes may be drawn into skin lesions and respiratory tract by local viral or bacterial infections. Secretion of interferon gamma by NK cells and γδ T lymphocytes may also contribute to the pathogenesis of the granulomatous lesions. A similar hypothesis has been suggested by Zimmer and colleagues on the basis of the
finding that NK cells from children with a deficiency in TAP2 were capable of lysing autologous B cells.2 3 21 The degree of self-reactivity of NK cells may perhaps differ for distinct clones and may be determined by the level of expression of HLA class-I molecules and the HLA haplotype of different patients.2 3

Expression of different HLA class-I haplotypes by different patients is likely to affect the range of their clinical symptoms and infections. Analysis of patients with defects in the class-I presentation pathway is therefore important if we are to understand the relation between susceptibility to infectious diseases, autoimmune disorders, and HLA class-I haplotype in an outbred population.

Contributors
H T M oins-Tisserenc and S D Gadol contributed equally to this work. H T M oins-Tisserenc characterised the biochemical and genetic defects of the patients and contributed to the writing of the paper. S D Gadola identified all the patients described in the manuscript, was involved in the biochemical analysis of some patients, and contributed to the writing of the paper. M Celli and M Colonna analysed the activity of NK and gammairadelta T cells in patient 3. P R Dunbar did the FACS analysis. A Eley identified patient 3. N Blake was involved in the presentation of viral antigens from patient 1. C Baycal, J Lambert, M Willemsen, P Bigiardi, and S Buchner were involved in the clinical analysis of patients 2, 3, 4, and 5. M Jones did immunohistochemistry on the tissue sections of patient 1. W G Ross clinically analysed patient 1, described her symptoms, and contributed to the writing of the paper. V Cerundolo directed this study, supervised all the experimental work, and contributed to the writing of the paper.

Acknowledgments
We thank K Weilh (Oxford T transplant Centre) for tissue typing the patients, A Kely (Cambridge) for analysis of the stability of HLA class-I molecules in the B cells from patient 1, S Fuggle, L Cerundolo (Nuffield Department of Surgery, Oxford), D M Aon G Racz (Division of Cellular Sciences, Oxford), and A-F Feller (Institute of Pathology, University of Lubec, Germany) for their help in analysing the biopsy samples; G M etors (Bloodtransfusionzentrum Antwerp HLA lab, Edegem) for tissue typing patient 4; W H Schmitt for assistance in the clinical characterisation of patient 1; and G Bird (Clinical Immunology, Churchill Hospital, Oxford) for advice and helpful discussion. This work was funded by the Medical Research Council of UK and by the Cancer Research Campaign. H T M oins-Tisserenc was supported by Fondation de France and S Gadola was supported by grants of the Swiss National Foundation, N ovaris Foundation, and Roche Research Foundation.

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