Major Histocompatibility Complex-independent Recognition of a Distinctive Pollen Antigen, Most Likely a Carbohydrate, by Human CD8^+ α/β T Cells

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Summary
We have isolated CD8^+ α/β T cells from the blood of atopic and healthy individuals which recognize a nonpeptide antigen present in an allergenic extract from Parietaria judaica pollen. This antigen appears to be a carbohydrate because it is resistant to proteinase K and alkaline digestion, is hydrophilic, and is sensitive to trifluoromethanesulphonic and periodic acids. In addition, on a reverse-phase high performance liquid chromatography column the antigen recognized by CD8^+ T cells separates in a fraction which contains >80% hexoses (glucose and galactose) and undetectable amounts of proteins. Presentation of this putative carbohydrate antigen (Pj CHO Ag) to CD8^+ T cell clones is dependent on live antigen presenting cells (APCs) pulsed for >1 h at 37°C, suggesting that the antigen has to be internalized and possibly processed. Indeed, fixed APCs or APCs pulsed at 15°C were both unable to induce T cell response. Remarkably, Pj CHO Ag presentation is independent of the expression of classical major histocompatibility complex (MHC) molecules or CD1. CD8^+ T cells stimulated by Pj CHO Ag-pulsed APCs undergo a sustained [Ca^{2+}]_i increase and downregulate their T cell antigen receptors (TCRs) in an antigen dose- and time-dependent fashion, similar to T cells stimulated by conventional ligands. Analysis of TCR Vβ transcripts shows that six independent Pj CHO Ag-specific T cell clones carry the Vβ8 segment with a conserved motif in the CDR3 region, indicating a structural requirement for recognition of this antigen. Finally, after activation, the CD8^+ clones from the atopic patient express CD40L and produce high levels of interleukins 4 and 5, suggesting that the clones may have undergone a Th2-like polarization in vivo. These results reveal a new class of antigens which triggers T cells in an MHC-independent way, and these antigens appear to be carbohydrates. We suggest that this type of antigen may play a role in the immune response in vivo.

It is well known that T lymphocytes expressing α/β TCR recognize antigenic peptide bound to MHC molecules. However, there is increasing evidence that nonprotein antigens can also be recognized by T cells (for review see reference 1). A first category of nonprotein antigens is represented by lipids or glycolipids such as mycolic acid and lipoarabinomannan (2–4). Presentation of these antigens to α/β T cells requires processing and is carried out by CD1 molecules which display a specificity for hydrophobic antigens (1). A second class of nonprotein antigens includes low mol wt phosphorylated molecules such as isopentenyl pyrophosphate and its synthetic analogs (5–7). In this case presentation to γ/δ T cells is independent from uptake and processing and does not require any known antigen presenting molecule.

A long-standing question is whether carbohydrates may be recognized by T cells. It has been shown that MHC molecules can bind glycopeptides via the peptide moiety and display the carbohydrate for interaction with TCR (8–11). However, this type of recognition still can be regarded as a variation of conventional peptide antigen recognition as it has been shown for haptenized peptide (12). So far, there has been neither evidence that carbohydrates may bind directly to MHC or other antigen presenting molecules (13, 14) nor reports that carbohydrates as such might trigger T cells in the absence of MHC molecules.

We have observed that T cells which proliferate in vitro...
in response to a pollen extract from *Paritaria judaica* (PJE), a common environmental allergen of the Mediterranean area (15), comprise a sizable fraction of CD8+ α/β T cells. Here we provide evidence that these T cells recognize a putative carbohydrate antigen present in PJE. Presentation of PJE Ag to T cells is dependent on APCs but is not restricted by either MHC or CD1 molecules. These results reveal a new mode of antigen presentation and a new class of ligands which can trigger α/β T cells.

**Materials and Methods**

PJE was kindly provided by Dr. G. De Libero, University of Naples, Italy. PJE was prepared by extracting PJE pollen (Allergon, Stockholm, Sweden) with 0.125 M bicarbonate buffer as described (16). The extract contained ~20% protein as assessed by the Bradford method (17). PJE was also purchased from Neo-Arpos (Malaga, Spain). To remove proteins from the extract two methods were used. First, 5 mg PJE was digested in 200 μl PBS, pH 7.2, with 100 μg proteinase K (PK; Sigma Chemical Co.) in the presence of 1 mM CaCl2 for 2 h at 37°C followed by heating to 65°C for 5 min. The digestion was repeated twice. Alternatively, 5 mg PJE was incubated in 100 μl 3 M NaOH/0.1 M NaBH4 overnight at room temperature. The samples (referred to as PJE and NaBH4PJE, respectively) were tested and compared to the same concentration of untreated or mock-treated PJE. To remove phosphates, PJE (2.5 mg) was treated with 10 U phosphatase A (Sigma Chemical Co.) in 100 μl of 50 mM Tris HCl, pH 8.3, for 2 h at 37°C. The enzyme was inactivated by heating to 65°C for 10 min. To remove sugars two methods were used. PJE (10 mg) was treated for 1 h at 0°C with trifluoromethane-sulfonic acid (TFMS) and anisole (1:2 ratio) as described (18). After incubation, an equal volume of ice-cold aqueous pyridine (60% vol/vol) was added. Alternatively, PJE (5 mg/ml) was incubated in 10 mM acetate buffer, pH 4.7, containing 10 mM sodium periodate (SP; Sigma Chemical Co.) for 6 h at 4°C (23). The reaction was stopped with a molar excess of ethylene glycol and NaBH4 was added at 2 mg/ml. The samples (referred to as TFMS PJE and SP PJE, respectively) were tested and compared to the same concentration of untreated or mock-treated PJE.

**Antigen Presentation Assay.** Cell lines were maintained in 10% FCS-RPMI (Hyclone Labs., Inc., Logan, UT). PBMCs from one atopic and one healthy individual were cultured in 96-well flat-bottomed plates (Costar Corp., Cambridge, MA) in RPMI 1640 supplemented with 2 mM L-glutamine, 1% nonessential amino acids, 1% pyruvate, 50 μg/ml kanamycin, 5 × 10⁻³ M 2-ME ( Gibco, Gaithersburg, MD) and 5% human serum (Sigma Chemical Co., St. Louis, MO) in the presence of PJE (50 μg protein/ml). Recombinant IL-2 (20 U/ml; provided by Dr. A. Lanzavecchia, Basel Institute for Immunology, Basel, Switzerland) was added at day 6. After 20 d, T cell blasts were cloned by limiting dilution. T cell clones were maintained by periodic restimulation with phytohemagglutinin, irradiated allogeneic PBMCs (3,000 rad from a 60Co source), and rIL-2 as previously described (16).

**Cell Lines.** Dendritic cells (DCs) were generated by culturing peripheral blood monocytes in the presence of GM-CSF and IL-4 (20). The extract contained ~10% protein as assessed by the Bradford method (21). PJE was also purchased from Neo-Arpos (Malaga, Spain). To remove proteins from the extract two methods were used. First, 5 mg PJE was digested in 200 μl PBS, pH 7.2, with 100 μg proteinase K (PK; Sigma Chemical Co.) in the presence of 1 mM CaCl2 for 2 h at 37°C followed by heating to 65°C for 5 min. The digestion was repeated twice. Alternatively, 5 mg PJE was incubated in 100 μl 3 M NaOH/0.1 M NaBH4 overnight at room temperature. The samples (referred to as PJE and NaBH4PJE, respectively) were tested and compared to the same concentration of untreated or mock-treated PJE. To remove phosphates, PJE (2.5 mg) was treated with 10 U phosphatase A (Sigma Chemical Co.) in 100 μl of 50 mM Tris HCl, pH 8.3, for 2 h at 37°C. The enzyme was inactivated by heating to 65°C for 10 min. To remove sugars two methods were used. PJE (10 mg) was treated for 1 h at 0°C with trifluoromethane-sulfonic acid (TFMS) and anisole (1:2 ratio) as described (22). After incubation, an equal volume of ice-cold aqueous pyridine (60% vol/vol) was added. Alternatively, PJE (5 mg/ml) was incubated in 10 mM acetate buffer, pH 4.7, containing 10 mM sodium periodate (SP; Sigma Chemical Co.) for 6 h at 4°C (23). The reaction was stopped with a molar excess of ethylene glycol and NaBH4 was added at 2 mg/ml. The samples (referred to as TFMS PJE and SP PJE, respectively) were tested and compared to the same concentration of untreated or mock-treated PJE.

**Purification of the Antigen Recognized by CD8+ T Cell Clones.** The antigen recognized by CD8+ T cell clones was purified from crude pollen extract using two different protocols. In the first protocol, PJE was dissolved in 4% trichloroacetic acid (15 mg/ml) and precipitated proteins were removed by centrifugation. The supernatant was neutralized by 1 N NaOH and lyophilized. The sample was fractionated on a preparative reverse-phase column (RP-8; Waters, Millipore Corporation, Bedford, MA) by eluting with a linear gradient of methanol in water. Collected fractions were concentrated at reduced pressure, lyophilized, and tested for biological activity as well as for carbohydrate and protein content. In the second protocol, PJE (300 mg in 80 ml water) was extracted (80:65, vol/vol aqueous to organic) with chloroform/methanol (2:1, vol/vol) and the two phases were separated by centrifugation. The upper layer and the interface were recovered and lyophilized, and the lower layer was dried under nitrogen. Most of the biological activity was found in the upper fraction. The water soluble material (92 mg) was dissolved in 12 ml water and separated using two cycles of centrifugal concentrators with a cutoff of 10 and 3 kD (Amicon Inc., Beverly, MA). The biological activity was attenuated by a factor of 100.
Pharmacia Biotech, Uppsala, Sweden) to eliminate unincorporated products were purified by means of a Microspin G50 column from the relevant clones was amplified for the V\textsubscript{\textbeta} sequence, direct sequencing was performed. The cDNA obtained was used to confirm that the clones contained only one genomic sequence. CDR3 spectratyping as previously described (26). Once the clones were identified, the cells were expanded with IL-2. After 20 d the cell lines were enriched for antigen-specific T cells as shown by a strong proliferative response to the inducing agent in the presence of autologous mononuclear cells (data not shown). Interestingly, in several independent cell lines from both individuals a sizable fraction of the T cells (20-30%) was CD8\textsuperscript{+} (Fig. 1A). Since it is unusual to find CD8\textsuperscript{+} T cells in cultures stimulated by protein antigens, we isolated a number of CD4\textsuperscript{+} and CD8\textsuperscript{+} cells from the atopic patient and the healthy individual (Fig. 1B) by limiting dilution and tested their capacity to recognize PjE.

Results

Isolation of CD8\textsuperscript{+} T cell clones specific for PjE. PBMCs from a PjE-sensitive patient and one nonatopic individual were stimulated in vitro with PjE. The responding cells were expanded with IL-2. After 20 d the cell lines were enriched for antigen-specific T cells as shown by a strong proliferative response to the inducing agent in the presence of autologous mononuclear cells (data not shown). Interestingly, in several independent cell lines from both individuals a sizable fraction of the T cells (20-30%) was CD8\textsuperscript{+} (Fig. 1A). Since it is unusual to find CD8\textsuperscript{+} T cells in cultures stimulated by protein antigens, we isolated a number of CD4\textsuperscript{+} and CD8\textsuperscript{+} cells from the atopic patient and the healthy individual (Fig. 1B) by limiting dilution and tested their capacity to recognize PjE.

As shown in Table 1, several CD8\textsuperscript{+} T cell clones isolated from the atopic patient and the healthy individual proliferated in response to PjE. Proliferation was specific since it was observed using different sources of PjE, while pollen extracts from unrelated species such as Cupressus arizonica and Olea europaea were ineffective (not shown). Interestingly, while CD8\textsuperscript{+} clones from the healthy control produced only IFN-\gamma, those isolated from the allergic patient produced high levels of IL-4 and IL-5 (Table 1) and expressed high levels of CD40L after stimulation (Fig. 1C), suggesting that they may have undergone a Th2-like polarization in vivo.

CD8\textsuperscript{+} T cell clones recognize a proteinase K-resistant, phosphatase-resistant, periodate-sensitive antigen. The nature of the antigen recognized by CD8\textsuperscript{+} PjE-specific T cell clones was investigated. The crude pollen extract was digested with proteinase K and subjected to alkaline hydrolys...
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sis. Both treatments destroyed proteins and abolished stimulation of PjE-specific CD4+ T cells, but did not affect and actually enhanced stimulation of the CD8+ clones, suggesting that CD8+ T cells recognize a nonprotein antigen (Fig. 2). Treatment with alkaline phosphatase did not decrease the capacity of PjE to stimulate CD8+ clones, indicating that the antigen does not contain critical phosphate residues (data not shown). When PjE was extracted with chloroform/methanol, the antigen recognized by CD8+ T cells separated in the aqueous phase (see below). Taken together, these properties suggested that the antigen recognized might be a carbohydrate. Therefore, we tested the sensitivity of the antigen to treatments which destroy sugars. In contrast to protease and phosphatase treatments, both periodic acid and TFMS treatment of PjE completely abolished recognition by CD8+ T cell clones, while the capacity to stimulate CD4+ T cell clones was not affected (Fig. 3). These results suggest that the antigen recognized by CD8+ T cell clones is not affected by removal of proteins or phosphate groups but is sensitive to treatment which degrades carbohydrates or related polyhydrate compounds.

Partial Purification of the Antigen Recognized by CD8+ T Cell Clones. In a first attempt to purify the antigen recognized by CD8+ T cell clones, we separated the material remaining after protein precipitation by a semipreparative reverse-phase column. The activity was recovered in several fractions and the most active was enriched in carbohydrates (83%) but still contained ~1% proteins (data not shown). In a second attempt, we combined phase fractionation with ultrafiltration and rpH PLC. After extraction with chloroform/methanol, the antigen recognized by CD8+ clones separated in the aqueous phase, which contains mainly polar molecules, but was absent at the interface and in the or-

| Donor A          | 3H-TdR (cpm × 10^3) | IL-4 (pg/ml) | IL-5 (pg/ml) | IFN-γ (pg/ml) | Donor B          | 3H-TdR (cpm × 10^3) | IL-4 (pg/ml) | IL-5 (pg/ml) | IFN-γ (pg/ml) |
|------------------|---------------------|--------------|--------------|--------------|------------------|---------------------|--------------|--------------|--------------|
| P4.2             | 30.5*               | 537‡         | 746‡         | >4,000*      | CP.27           | 23.4               | <20         | <20         | 2,395        |
| P4.4             | 23.5                | <20          | <20          | >4,000       | CP.28           | 18.7               | 35          | <20         | >4,000       |
| P4.6             | 33.9                | 222          | 959          | >4,000       | CP.31           | 14.6               | <20         | <20         | 3,000        |
| P4.15            | 27.3                | 356          | <20          | >4,000       | CP.38           | 25.6               | <20         | <20         | 2,610        |
| P4.16            | 23.9                | 232          | <20          | 2.821        | CP.44           | 10.7               | ND          | ND          | ND           |
| P4.17            | 42.3                | 80           | 543          | >4,000       | CP.45           | 7.4                | ND          | ND          | ND           |
| P4.26            | 44.3                | 1,378        | 1,300        | 3,394        | CP.53           | 26.0               | ND          | ND          | ND           |
| P4.27            | 29.0                | 664          | 319          | >4,000       | CP.56           | 10.0               | ND          | ND          | ND           |
| P4.33            | 30.3                | 518          | >2,000       | 2,624        | CP.66           | 27.8               | <20         | <20         | >4,000       |
| P4.52            | 36.1                | 162          | <20          | 3,359        | CP.68           | 69.2               | 102         | <20         | >4,000       |

* T cells were cultured with PjE (50 μg/ml) and autologous B-LCL. Proliferative response was measured on day 3. Background response in the absence of antigen for all clones was <10^3.

† T cells were stimulated with 10^-7 M PMA + 0.2 μg/ml mAb TR66 (anti-CD3). After 40 h supernatants were collected and tested for cytokines by ELISA.

Figure 2. CD8+ T cell clones recognize a proteinase K (PK)-resistant, alkaline-resistant antigen of PjE. CD8+ T cell clones P4.2 (A) and CP.68 (C) and CD4+ T cell clones P6.2 (B) and CP.39 (D) were stimulated with autologous irradiated B-LCL in the presence of different concentration of PjE (○), PjE (●), or PjE (■). A mock control for proteinase K (○) was also included. Proliferative response was measured on day 3. Digestion with pronase gave comparable results (not shown).
Organic phase (data not shown). The hydrophilic fraction was separated using centrifugal concentrators with cutoff of 10 and 3 kD. The biological activity was recovered in the filtrate of the 10-kD concentrator and was partially retained in the 3-kD concentrator. The 3-kD filtrate was further fractionated on an rPHPLC by elution with a gradient of methanol in water (Fig. 4 A). As expected, the material with biological activity eluted very early while most of the contaminating compounds still present in the 3-kD filtrate were retained by the column and were eluted at later time points (Fig. 4 B and data not shown). The positive fraction was ~20-fold more active on a weight basis than the starting material (Fig. 4 B, inset). When this fraction was analyzed by GC-MS, the chromatographic elution and the mass spectrum revealed the presence of glucose and galactose at a molar ratio of 47:53 (Fig. 4 C). Furthermore, the same fraction contained no detectable peptides as assayed by the Bradford method (17), direct and reverse-phase TLC of the hydrolyzed fraction (HCl 6 N, 110°C, 6 h) followed by ninidrin staining, Edman degradation, and amino acid analysis using a 494 protein sequencer (data not shown). To further exclude any peptide contamination, the active fraction was digested with carboxypeptidase Y, a protease which digests most peptides including those with blocked NH2 termini (27). This treatment did not affect the bioactivity of the purified antigen but completely abolished the ability of a tetanus toxoid peptide to stimulate a specific CD4+ T cell clone (data not shown). Taken together, the above results suggest that, according to two different criteria (i.e., sensitivity to chemical treatment and purification with two methods), the antigen recognized by CD8+ clones is a low mol wt polyhydrate molecule, possibly a carbohydrate.

A PC-dependent and MHC- and CD1-independent Presentation of PjCHOAg to CD8+ T Cell Clones. The requirements for antigen recognition were studied using as readout T cell proliferation and cytokine production. The CD8+ clones proliferated in response to PjE only in the presence of APC (Fig. 5 A). Optimal presentation was provided by B-LCL and PBMCS, while, surprisingly, DCs were considerably less efficient (Fig. 5, A, and B). Several unrelated B-LCL tested were able to function as APCs for the CD8+ clones (Fig. 5 A), even if they lacked class I (221 and C1R) or class II molecules (SJO and T2) (Fig. 5 C). In addition, antibodies to class I and class II molecules did not inhibit T cell activation (Fig. 5 D), while they did inhibit antigen recognition by class I- and class II-restricted T cell clones (data not shown). A contribution of CD1 molecules was also ruled out by the finding that (a) anti-CD1 antibodies did not affect the proliferative response and (b) CD1-a, -b, and -c transfectants were as efficient as the untransfected control (data not shown). Taken together, these data suggest that none of the known antigen-presenting molecules are involved in the presentation of PjCHOAg to CD8+ α/β T cell clones.

The requirements for antigen uptake and processing were studied using [Ca2+]i increase as a sensitive and rapid assay of T cell antigen recognition. No response was observed when the CD8+ T cells were incubated with the antigen in solution, confirming the requirements for APCs (Fig. 6 D). In contrast, the clones showed a strong and sustained [Ca2+]i increase when conjugated with PjE-pulsed APC. Indeed, a time course of antigen pulsing revealed that a short incubation with antigen (1 h) was not sufficient for presentation (Fig. 6 B) and that the stimulatory capacity was progressively acquired during the next 5 h of incubation (Fig. 6 C). While APC fixed after antigen pulse retained the ability to induce [Ca2+]i increase in T cell clones (data not shown), APC fixed before a 5-h pulse at 37°C with PjE were ineffective (Fig. 6 E). In addition, live APC pulsed at 15°C failed to stimulate T cells (Fig. 6 F). Interestingly, chloroquine did not inhibit presentation of PjCHOAg (data not shown), suggesting that processing in an acidic

Figure 3. Oxidation with periodic acid and treatment with TFMS completely abolished recognition of PjE by CD8+ T cell clones. CD8+ T cell clones P4.2 (A) and P6.8 (C and E) and CD4+ T cell clones P6.2 (B) and CP.39 (D and F) were cultured with autologous irradiated B-LCL in the presence of different concentration of PjE (○), 110°/PjE (●), or 3PjE (■). Proliferative responses were measured on day 3. A mock control without periodate gave comparable results as PjE (data not shown).

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compartment was not involved. Finally, APC retained the capacity to stimulate T cells after removal of the antigen (Fig. 6, G–I). By comparing the stimulatory capacity of APC chased for different times with that of APC pulsed with different concentrations of antigen, we estimated the half-life of antigen on pulsed APC to be \( \approx 1 \) h. Taken together these results suggest that PjCHOAg has to be captured by the APC and it is displayed on their surface in a stable compartment was not involved. Finally, APC retained the capacity to stimulate T cells after removal of the antigen (Fig. 6, G–I). By comparing the stimulatory capacity of APC chased for different times with that of APC pulsed with different concentrations of antigen, we estimated the half-life of antigen on pulsed APC to be \( \approx 1 \) h. Taken together these results suggest that PjCHOAg has to be captured by the APC and it is displayed on their surface in a stable compartment was not involved. Finally, APC retained the capacity to stimulate T cells after removal of the antigen (Fig. 6, G–I). By comparing the stimulatory capacity of APC chased for different times with that of APC pulsed with different concentrations of antigen, we estimated the half-life of antigen on pulsed APC to be \( \approx 1 \) h. Taken together these results suggest that PjCHOAg has to be captured by the APC and it is displayed on their surface in a stable compartment was not involved. Finally, APC retained the capacity to stimulate T cells after removal of the antigen (Fig. 6, G–I). By comparing the stimulatory capacity of APC chased for different times with that of APC pulsed with different concentrations of antigen, we estimated the half-life of antigen on pulsed APC to be \( \approx 1 \) h. Taken together these results suggest that PjCHOAg has to be captured by the APC and it is displayed on their surface in a stable
fashion, possibly associated with molecules preferentially expressed on B cells.

TCR-β Usage and Evidence for the Involvement of TCR in the Recognition of PiCHOAg. All CD8⁺ PiCHOAg-specific T cell clones isolated from two individuals express the Vβ8 chain as detected by mAbs. This finding is compatible with stimulation by a Vβ8-specific superantigen possibly present in the allergenic extract. However, this possibility was ruled out by the finding that polyclonal Vβ8⁺ lines were not stimulated by PiE (data not shown). Since we could exclude a superantigen type of recognition we searched for a conserved motif in the CDR3 region (Table 2). Sequencing of the Vβ chains of six independent T cell clones revealed a remarkable conservation in positions 3 and 5 of the CDR3 region (threonine and valine, respectively), while the length differed by only one amino acid. In addition, all six clones analyzed used the Jβ1.2 segment. We also investigated the kinetics of T cell activation by PiCHOAg. As shown in Fig. 7, CD8⁺ T cells exposed to PiE-pulsed APC undergo a [Ca²⁺] increase that is sustained for >30 min (Fig. 7 A) and downregulate their TCRs in an antigen dose- and time-dependent fashion (Fig. 7 B and data not shown). These results demonstrate that PiCHOAg triggers the TCR with the same kinetics as conventional TCR ligands such as peptide-MHC complexes.

Discussion

We have shown that CD8⁺ α/β T cells can specifically recognize a carbohydrate antigen present in a pollen extract. This antigen differs from other nonpeptide antigens (2–7, 28) because of its chemical nature and the lack of MHC or CD1 restriction.

Several lines of evidence suggest that the stimulatory ligand is a carbohydrate. First, it is resistant to treatments that destroy proteins, such as proteinase K digestion and alkaline treatment, excluding a possible contribution of a peptide. Second, it separates in the aqueous phase, ruling out the possibility that it may contain lipids. Third, it is sensitive to agents that destroy polysaccharides, such as
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TFMS and periodic acid. Finally, it is enriched in a fraction containing high levels of glucose and galactose. However, we cannot exclude that other polyhydrate molecules with similar sensitivity to chemical treatments may be responsible for the stimulation of PjCHOAg-specific T cell clones. Although the structure of the epitope still has to be defined, it is likely to be intracellularly generated since we have found that, in order to stimulate specific T cells, PjCHOAg has to be pulsed with live APC for >1 h. Indeed, fixed APC or APC pulsed at 15°C were both unable to induce T cell response. We have found that chloroquine does not interfere with presentation of PjCHOAg, suggesting that either processing of this antigen is not required or may involve a different intracellular compartment. More evidence is needed to elucidate these points.

A remarkable finding is that recognition of PjCHOAg, unlike recognition of other ligands by α/β T cells, does not require the expression of classical MHC class I, class II, and CD1 molecules. However, once generated the epitope remains associated with the cell surface with a half life of ~1 h. We envisage two possibilities to explain how this epitope is displayed on APC surface for T cell recognition. First, the antigen may form a complex with a nonpolymorphic molecule which functions as classical antigen-presenting molecule, i.e., which bind antigen and interact with TCR. Second, PjCHOAg may be directly displayed on the cell membrane alone or in association with an antigen-binding molecule that serves only to concentrate the antigen but does not interact with TCR. Interestingly, DCs, which are the most efficient APC for protein antigens (29), are rather ineffective in presenting PjCHOAg, suggesting that they may lack this putative antigen-presenting/binding molecule.

Recognition of PjCHOAg by CD8+ T cell clones is mediated via TCR as shown by the kinetics of TCR triggering which is comparable to that of conventional TCR ligands such as peptide–MHC complexes (30). Moreover the presence of a conserved motif in the CDR3 region of the Vβ chain suggests the existence of a precise structural require-

Table 2. Nucleotide and Predicted Amino Acid Sequences of TCR-β Chains from PjCHOAg-specific CD8+ T Cell Clones from Two Different Donors

| Clone | Vβ | CDR3 | Jβ |
|-------|----|------|----|
| Donor A |   |      |    |
| P4.2  | 8  | CASSPGTGVDGYTF | 1.2 |
|       |    | tgtgccagtacctgcgcgcgcggtgagctacaccttc | |
| P4.17 | 8  | CASSLVTGVDGYTF | 1.2 |
|       |    | tgtgccagtacctgcgcgcggtgagctacaccttc | |
| P4.52 | 8  | CASSQATAVNGYTF | 1.2 |
|       |    | tgtgccagtacctgcgcgcggtgagctacaccttc | |
| Donor B |   |      |    |
| CP.31 | 8  | CASSVATAVCTCGYTF | 1.2 |
|       |    | tgtgccagtacctgcgcgcggtgagctacaccttc | |
| CP.38 | 8  | CASSGTSVAMGYTF | 1.2 |
|       |    | tgtgccagtacctgcgcgcggtgagctacaccttc | |
| CP.68 | 8  | CASSVATAVTDGYTF | 1.2 |
|       |    | tgtgccagtacctgcgcgcggtgagctacaccttc | |

CDR3 regions run from the consensus cysteine to the first phenylalanine of the “FGXT” motif found in the J region. Identity of Dβ segments was assigned when at least six consecutive bases identical to those of a germline Dβ segment were identified within the junctional region.

Figure 7. PjCHOAg triggers the TCR as conventional TCR ligand. (A) Time course of [Ca2+]i increase in T cell clone P4.2 conjugated with PjE-pulsed B-LCL. (B) TCR downregulation in T cells conjugated with unpulsed APC or PjE-pulsed APC.
ment for PjCHOAg recognition (30). This result, together with the fact that polyclonal Vβ8+ lines do not respond to PjCHOAg, rules out the possibility that PjCHOAg may act as a superantigen.

The finding that CD8+ T cell clones could be isolated from both atopic patients and from healthy donors is not surprising since they both are naturally exposed to this environmental allergen (31). However, it is interesting that only the clones from the atopic patient produce high levels of IL-4 and IL-5 and express CD40L, suggesting that these clones may function as typical carrier-specific T cells. Interestingly, these clones are not cytotoxic (Sallusto, F., unpublished data) and may thus provide cognate help to antigen-specific B lymphocytes. If this is a general case, the polarization of the specific CD8+ cells towards help or cytotoxicity may become a critical factor controlling the stimulation of an IgE response or its suppression (38, 39).

These results suggest that the response to carbohydrate antigens may be part of the normal response to environmental allergens and that this response can be polarized in atopic patients. Polysaccharides are generally considered typical T-independent antigens. However, T cells that modulate responses to polysaccharides have been observed in several systems (40–46). Our results might explain these earlier observations and raise the possibility that T cell recognition of carbohydrates may play a role in the immune response.

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