EXTENDED REPORT

Pars planitis is associated with an increased frequency of effector-memory CD57+ T cells

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Br J Ophthalmol 2007;91:1393–1398. doi: 10.1136/bjo.2007.116277

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Accepted 25 April 2007
Published Online First
2 May 2007

Aim: To evaluate the frequency, phenotype and the potential function of CD57+ T cell subsets in patients with pars planitis.

Methods: CD4+CD57+ and CD8+CD57+ T cells were quantitated in peripheral blood from 15 patients with pars planitis and 15 healthy controls. To evaluate the phenotype and potential function of CD57+ T cell subsets CCR7, CD27, CD28, CD45RA, CD45RO, intracellular IFN- γ , IL-4, perforin and granzyme-A expression were assessed by flow cytometry.

Results: CD57+ T cells subsets were increased in patients with pars planitis ($p=0.002$). The majority of CD4+CD57+ T cells were CCR7–CD27–CD28–CD45RO+, while the most CD8+CD57+ T cells were CCR7–CD27–CD28–CD45RA+. The number of cells positive for intracellular IFN- γ and IL-4 was higher in the CD57+ T cell populations. A greater number of CD8+CD57+ T cells than CD8+CD57– T cells were positive to perforin ($p=0.006$) and granzyme-A ($p=0.01$).

Conclusions: CD57+ T cells had a phenotype associated with peripheral memory (CCR7–CD27–CD28–). Cytokine production by CD57+ T cells suggests that these cells may play a role in helper cell regulation. High expression of intracellular proteins involved in cytotoxicity suggests that CD8+CD57+ T cells may play an effector role. Taken together, this study proposes that CD57+ T cells function as memory-effector T cell subsets during pars planitis pathogenesis.

Pars planitis is an idiopathic intermediate uveitis characterised by chronic and insidious intraocular inflammation involving anterior vitreous, peripheral retina, and pars plana. While the cause of pars planitis remains unknown, CD4+ T cells expressing the activation marker CD69 in either peripheral blood¹ or the aqueous humor of patients with idiopathic uveitis² support the hypothesis that T-helper cells are involved in inducing and sustaining the inflammatory process. Little is known about the role of CD8+ T cells in pars planitis however, although CD8+CD56+ T cells are associated with Behçet's uveitis³. This issue is of particular interest because coexpression of functional NK receptors, like CD56 or CD57 on CD8+ T cells, is thought to be associated with effector function.^{3–4} In particular, CD57 expression on both CD4+ and CD8+ T cells, correlates with late immune responses⁵ and some pathologic conditions resulting from persistent stimulation of the immune system, including HIV, tuberculosis, colorectal cancer and arthritis rheumatoid.^{6–10} The aim of this work was to study the frequency, phenotype and potential function of CD57+ T cells subsets during a chronic eye disease such as active pars planitis.

MATERIALS AND METHODS

Patients

Thirty individuals (19 males and 11 females, mean age 9.5 years, range 6–14) with idiopathic pars planitis were studied. Pars planitis diagnosis was based on clinical history (mean disease duration 4 (SD 2) years), eye and physical examination. All patients were classified as having active intermediate uveitis according to the diagnostics standards of the SUN working group.¹¹ Healthy age- and sex-matched volunteers were used as controls. All participants gave their informed consent for blood sampling after written information was provided. This study adhered to the tenets of the Declaration of Helsinki and was approved by the Medical Investigation and Bioethics

Committees of the Institute of Ophthalmology "Fundacion Conde de Valenciana" in Mexico City.

Monoclonal antibodies and reagents

Phycoerythrin-labelled mouse IgG monoclonal antibodies (mAbs) against human CD28, IL-4, granzyme-A, and FITC-labelled antibodies against human CD57, IFN- γ , perforin, were purchased from BD PharMingen (San Diego, CA, USA). PE-labelled mAbs against human CD57 were purchased from Santa Cruz Biotechnology, Inc (Santa Cruz, CA, USA). FITC-labelled goat anti-mouse IgM, FITC-labelled mouse anti-human CD4 and CD45RA and phycoerythrin-labelled mouse anti-human CD4 and CD45RO mAbs were purchased from Southern Biotech Inc (Birmingham, AL, USA). FITC-labelled mAbs against human CD27, and phycoerythrin-Cy5-labelled mAbs anti-CD8 and CD4, were obtained from e-Biosciences (San Jose, CA, USA). FITC- and phycoerythrin-labelled mAbs anti-human CCR7 were purchased from RD Systems (Minneapolis, MN, USA). Lymphoprep (Ficoll 1.077 density) was obtained from Nycomed Pharma (Nyegaard, Oslo, Norway). Saponin, brefeldin-A, RPMI-1640 culture medium, PMA, ionomycin, and salts were purchased from Sigma Chemical Co (St Louis, MO, USA). Sodium pyruvate, L-glutamine, and 2-mercaptoethanol were obtained from Gibco BRL (Rockville, MD, USA). Fetal calf serum was purchased from HyClone Labs (Logan, UT, USA).

Peripheral blood mononuclear cells

Whole heparinised peripheral blood was diluted 1:2 (vol/vol) in phosphate buffered saline (PBS), pH 7.2. Peripheral blood mononuclear cells (PBMCs) were separated on a Ficoll density gradient by centrifugation at 1700 rpm for 30 min at room temperature. After centrifugation, the interface cells were

Abbreviations: PBMC, peripheral blood mononuclear cell; PBS, phosphate buffered saline

Figure 1 Expression of CD4, CD8 and CD57 on peripheral blood mononuclear cells (PBMC). (A) CD4 and CD57 expression on PBMC; (B) CD8 and CD57 expression on PBMC. Representative images of one of 15 patients with pars planitis and one of 15 healthy controls.

collected, washed twice, and counted using a haemocytometer to assess viability by trypan blue dye exclusion.

Immunofluorescence staining of cell surface markers

Triple-colour staining was performed on PBMC by direct immunofluorescence, using FITC- or phycoerythrin-mAb anti-CD57 and phycoerythrin-Cy5-labelled mAbs against human CD4 or CD8, and either FITC- or phycoerythrin-labelled mAbs against CD27, CD28, CD45RA, CD45RO or CCR7. Briefly, 2×10⁵ cells were suspended in 20 µl PBS supplemented with 0.2% bovine serum albumin and 0.2% sodium azide (PBA), and incubated with fluorochrome-labelled mAb for 30 min at 4°C. After incubation, the cells were washed twice with PBA, fixed with 1% *p*-formaldehyde and analysed by flow cytometry.

Cell cultures

PBMC were cultured in 24-well flat bottomed cell culture plates (Costar, Cambridge, MA, USA) at 1×10⁶ cells/well in RPMI-1640 medium supplemented with 1 mM sodium pyruvate, 2 mM L-glutamine, 50 µg/ml gentamicin and 0.5% heat-inactivated fetal calf serum, and incubated at 37°C in a 5% CO₂ humidified chamber. After 24 h the culture medium was

removed and fresh culture medium supplemented with 10% heat-inactivated fetal calf serum, PMA/ionomycin (5 ng/ml–0.2 µg/ml) and brefeldin-A (10 µg/ml) were added. Four hours later, the cells were harvested and processed to measure intracellular protein expression by flow cytometry.

Immunofluorescence staining of intracellular proteins

Stimulated or unstimulated PBMCs were washed with PBA and stained with fluorescent labelled mAbs against CD57 and either CD4 or CD8 for 30 min. After washing, the cells were fixed with 4% *p*-formaldehyde in PBS for 10 min at 4°C. The cells were washed twice with PBS and permeabilised with saponin buffer (0.1% saponin and 10% BSA in PBS) by shaking gently for 10 min at room temperature. The cells were then incubated with phycoerythrin-labelled mAbs against human IL-4 or granzyme A, and FITC-labelled anti-human IFN-γ or anti-perforin antibodies. In all cases isotype-matched Ig/FITC and Ig/ phycoerythrin controls were used. After 30 min the cells

Table 1 Frequency of cells analysed in lymphocyte gate		
	Patients with pars planitis	Healthy controls
CD4+	35 (5)	32 (7)
CD8+	20 (2)	25 (2)
CD4+CD57+	6 (3)*	3 (1)*
CD8+CD57+	15 (3)†	3 (1)†

Mean (standard error), n = 15.
The cells are represented in percentage of lymphocyte gated cells.
CD4+ and CD8+ are total percentage of lymphocyte gated cells.
*p = 0.01; †p = 0.003.

Table 2 Phenotypic characterisation of CD57+ and CD57– T cells in patients with pars planitis				
	CD4+		CD8+	
	CD57–	CD57+	CD57–	CD57+
CD27	53.2 (11)*	17.6 (6)*	55.6 (5)**	21.5 (2)**
CD28	83.5 (10)*	54.2 (9)*	54.7 (8)††	24.3 (6)††
CD45RA	52.5 (6)‡, §	19.5 (6)‡, †	60.4 (7)‡‡	67.2 (8)§§
CD45RO	28.2 (3)‡, §	72.3 (8)‡, †	19.7 (5)‡‡	23.0 (5)§§
CCR7	52.5 (11)¶	16.0 (8)¶	46.4 (4)¶¶	1.2 (1)¶¶¶

Mean (standard error), n = 15.
*p = 0.02; †p = 0.0005; ‡p = 0.002; §p = 0.009; ¶p = 0.03; **p = 0.001; ††p = 0.01; ‡‡p = 0.0003; §§p = 0.0001; ¶¶p < 0.001.

Figure 2 Phenotypic characterisation of CD4+CD57- and CD4+CD57+ T cells. Representative histograms of effector-memory related markers on CD57- and CD57+ T helper subsets in patients with pars planitis.

were washed with PBS, fixed again with 1% *p*-formaldehyde and analysed by flow cytometry.

Flow cytometric analysis

All cells were analysed for marker expression by collecting 5000 events using a FACScan flow cytometer (Becton Dickinson, CA, USA) and CellQuest Pro software. To analyse cell surface marker staining, a gate was drawn around the lymphocyte population based on their physical properties (forward and side scatter) and a second gate was drawn based on positive fluorescent antibody binding to CD4 and CD8. To analyse intracellular protein staining, positive and negative fluorescence staining of CD57+ T cells (forward scatter and fluorescence) were set manually based on the distribution of cells stained with the isotype controls. Data are presented as two-dimensional dot plots or histograms. Control stains were performed using isotype-matched mAb of unrelated specificity that were labelled with FITC-, phycoerythrin-Cy5- or phycoerythrin. Background staining was <1% and was subtracted from experimental values.

Statistical analysis

T tests or Mann-Whitney U tests were used to detect significant differences. The analysis was performed with Sigma-Stat 3.1 and Sigma-Plot 10.0 software. Differences were considered statistically significant when the test yielded *p* values less than 0.05.

Figure 3 Phenotypic characterisation of CD8+CD57- and CD8+CD57+ T cells. Representative histograms of effector-memory related markers on CD57- and CD57+ T CD8+ cells in patients with pars planitis.

RESULTS

Increased frequency of CD57 T cells subsets in patients with pars planitis

We began by determining the percentage of CD57+ T cells in the peripheral blood of 15 patients with pars planitis and 15 healthy controls. While the percentage of CD4+ and CD8+ T cells was similar between both groups, the frequency of CD4+CD57+ T cells was 2.2 times higher in patients with pars planitis than healthy control individuals ($p = 0.01$). In contrast the frequency of CD8+CD57+ T cells was 5.3 times higher in patients with pars planitis than healthy controls ($p = 0.0003$) (fig 1, table 1).

CD57 expression on differentiated effector memory T cell subsets

To determine whether CD57 was associated with central memory or effector memory T cell subsets,¹² we measured CD27, CD28, CD45RA, CD45RO and CCR7 expression on CD57+ and CD57- T cell subsets from 15 patients with active pars planitis. The vast majority of CD4+CD57+ had 2.6-fold less expression of CD27 ($p = 0.003$) (82.4 (SE 6) were CD27-) and 5.2 times lower expression of CCR7 ($p = 0.0001$) (84 (SE 8) were CCR7-) than CD4+CD57- T cells. Helper CD57+ T cells also showed 1.5-fold less expression of CD28 than CD57- T cells ($p = 0.02$). The percentage of CD4+CD57+ T cells expressing CD45RO was 2.5 times higher than the percentage of CD4+CD57- T cells expressing this marker ($p = 0.002$). In

contrast, CD45RA expression was 2.6-fold higher on CD4+CD57[−] T cells than CD4+CD57⁺ T cells ($p = 0.0005$) (fig 2, table 2).

CD8+CD57⁺ T cells showed reduced expression of CD27 ($p = 0.0009$) (76.5 (SE 5) were CD27[−]) and 2.8-fold reduced expression of CD28 ($p = 0.004$) (73.9 (SE 6) were CD28[−]) than CD8+CD57[−] T cells. Furthermore, 42.6-fold fewer CD8+CD57⁺ T cells expressed CCR7 ($p < 0.001$) (97.7 (SE 1) were CCR7[−]) than CD8+CD57[−] T cells (fig 2, table 2). In contrast to helper CD57⁺ T cells, CD8⁺ T cells were predominantly CD45RA⁺, independent of their CD57 status (fig 3, table 2).

Intracellular IFN- γ and IL-4 are more highly expressed on CD57⁺ than CD57[−] T cells

Polyclonal stimulation *in vitro* showed that CD57⁺ T cells were able to produce cytokines. As shown in table 3, IFN- γ was expressed by 11.8-fold more in CD4+CD57⁺ T cells than CD4+CD57[−] T cells ($p < 0.001$). Similarly, IL-4 was expressed by 8.4-fold more in CD4+CD57[−] T cells than CD4+CD57[−] cells ($p < 0.001$) (fig 3, table 3). Likewise, IFN- γ was expressed by 3.9 more in CD8+CD57⁺ T cells in comparison to CD8+CD57[−] T cells. Interestingly, CD8+CD57⁺ T cells produced 2.6 times more IFN- γ than IL-4 ($p = 0.002$) (fig 4, table 3).

Perforin and granzyme-A expression on CD57⁺ and CD57[−] CD8⁺ T cells

To establish the potential functional involvement of the granule-exocytosis pathway in CD57⁺ and CD57[−] T cells, we assessed the percentage of CD8⁺ T cells containing perforin and granzyme-A. Histogram analysis showed that CD8+CD57⁺ T cells expressed 13.5 times more perforin, and 8.2 times more granzyme-A than CD8+CD57[−] T cells ($p < 0.001$) (fig 5, table 4).

DISCUSSION

Systemic CD4+CD69⁺ T cells responses are associated with idiopathic intermediate uveitis¹ a chronic and insidious eye disease. Numerous studies have associated CD57 expression with persistent antigenic stimulation and chronic diseases.^{6–10} In this study, we characterised a population of CD57⁺ T cells

Table 3 Intracellular cytokine expression by CD57 ⁺ and CD57 [−] T cells				
	CD4 ⁺		CD8 ⁺	
	CD57 [−]	CD57 ⁺	CD57 [−]	CD57 ⁺
IFN- γ	5.5 (1)*	64.8 (4)*,†	6 (2)*,‡	23.4 (2)*
IL-4	6.0 (3)*	51.0 (6)*,†	1 (0.2)‡,*	9.0 (2)‡

Mean (standard error), $n = 15$.
* $p < 0.001$; † $p = 0.02$; ‡ $p = 0.002$.

Table 4 Perforin and granzyme-A expression by CD57 ⁺ and CD57 [−] T cells		
	CD8 ⁺ T cells	
	CD57 [−]	CD57 ⁺
Perforin	3.5 (2)*	46.3 (4)*
Granzyme-A	10.5 (3)*	84.3 (6)*

Mean (standard error), $n = 15$.
* $p < 0.001$.

that produced cytokines and contain granules associated with perforin-granzyme A cytotoxic pathway, and showed increased frequency of this population in patients with pars planitis. As previously reported,⁶ these cells were also found to express an effector/memory phenotype with reduced levels of CCR7, CD27 and CD28. CCR7 is a cysteine chemokine receptor that controls homing to secondary lymphoid organs and divides human memory T cells into two functionally distinct subsets CCR7[−] and CCR7⁺ T cells. CCR7[−] cells are memory cells that migrate to inflamed tissues and display immediate effector function (effector-memory), while CCR7⁺ cells are memory cells that express lymph-node homing receptors and lack immediate effector function (central-memory).¹³ Likewise, the absence of CD27 and CD28 expression on T cells is associated with cellular memory or effector characteristics. Fritsch RD *et al*¹⁴ char-

Figure 4 Comparative histogram analysis of intracellular cytokines on CD57[−] and CD57⁺ T cells subsets. The x axis denotes the fluorescence intensity to IFN- γ or IL-4 on CD57[−] T cells (thin line) and on CD57⁺ T cells (thick line) on both CD4 gated cells (top) and CD8 gated cells (bottom).

Figure 5 Perforin and granzyme-A expression on CD8+CD57[−] and CD8+CD57⁺ T cells. Representative histograms of perforin and granzyme-A expression on CD57 subsets. The x axis denotes the fluorescence intensity to granzyme-A (top) or perforin (bottom) on CD57[−] T cells and on CD57⁺ T cells.

acterised three distinct memory CD4 subpopulations that are distinguished by expression of CCR7 and the TNFR family member CD27. Telomere length also differed in these subsets (CCR7+CD27+ > CCR7[−]CD27+ > CCR7[−]CD27[−]), suggesting that CCR7[−]CD27[−] cells are more differentiated than CCR7[−]CD27+ cells. Tomiyama H *et al* showed that CD8⁺ T cells expressing CD27[−]CD28[−]CD45RA^{+/−} phenotype are classified as effector cells.¹⁵ As previously suggested, the vast majority of CD4+CD57⁺ T cells had memory phenotype characterised by CD45RO expression.⁷ In contrast CD8+CD57⁺ T cells were predominantly CD45RA. The CD45RA isoform is expressed on naive T cells and a subset of effector cells and it has been proposed that CD45RA memory cells (CD28[−]CD27[−]CCR7[−]) represent the most differentiated memory cells.^{12 16 17}

PBMCs from patients with sarcoid intermediate uveitis are polarised towards a Th1 response.¹ Thus we measured intracellular cytokine expression in CD57⁺ T cells following activation with a polyclonal stimulus. Our results demonstrated a significant increase in the frequency of CD57⁺ T cells expressing either IFN- γ or IL-4. Th1 polarisation was only observed in the CD8+CD57⁺ T cell subset (Tc1 response). Interestingly, some findings have suggested that idiopathic intermediate uveitis is not associated with a Th1 immune response¹ while others have shown a systemic Th1 response is found in both Behçet's uveitis and idiopathic intermediate uveitis.¹⁸ Our results support the hypothesis about Th1 polarisation. However it is critical to measure the levels of other regulatory cytokines to better define the real polarity of these cell subsets.

CD8⁺ T cell effector function is also defined by the expression of cytotoxic granules like perforin and granzyme-A.¹⁹ These cytotoxic granules influence T cell-mediated effector function against endogenous antigens.²⁰ Interestingly, we found that a significant number of CD57⁺ T cells expressed perforin and granzyme A as shown by Sada-Ovalle *et al*,²¹ supporting the notion that CD8+CD57⁺ T cells are effector cells.

The increase in frequency of CD57⁺ T cells has already associated to systemic infections^{6 21} and autoimmune diseases such as rheumatoid arthritis.^{10 22 23} Hingorani R *et al*²² demonstrated that CD8+CD57⁺ T cells from patients with rheumatoid arthritis were clonally expanded in the synovial fluid. Tan LC *et al*¹⁰ confirmed this data, but they showed specificity for viral antigens and not for auto-antigens. Although it is not clear if

these virus-specific T cells play a role in the maintenance of local inflammation, Le Priol Y *et al*²³ recently suggested that CD8+CD57⁺ cells from HIV-infected individuals are destined to migrate to non-lymphoid tissues to exert its cytotoxic activities. In this regard, it will be interesting to analyse if CD57 expansion is related to infection in patients with pars planitis.

In summary, our results suggest that the CD57⁺ T cells are terminally differentiated cell subsets that probably act as memory-effector T cells during pars planitis pathogenesis. Whether CD57⁺ T cell subsets are directly involved in the eye inflammation is still unknown and needs further investigation.

ACKNOWLEDGEMENTS

The authors thank Verónica Romero Martínez for technical assistance.

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Funding: This work was supported by a grant from National Council of Science and Technology (CONACYT-SALUD 2005-14108).

Competing interests: None.

REFERENCES

- Murphy CC, Duncan L, Forrester JV, *et al*. Systemic CD4(+) T cell phenotype and activation status in intermediate uveitis. *Br J Ophthalmol* 2004;**88**:412–16.
- Calder VL, Shaer B, Muhaya M, *et al*. Increased CD4⁺ expression and decreased IL-10 in the anterior chamber in idiopathic uveitis. *Invest Ophthalmol Vis Sci* 1999;**40**:2019–24.
- Ahn JK, Chung H, Lee DS, *et al*. CD8brightCD56⁺ T cells are cytotoxic effectors in patients with active Behçet's uveitis. *J Immunol* 2005;**175**:6133–42.
- Tarazona R, DelaRosa O, Alonso C, *et al*. Increased expression of NK cell markers on T lymphocytes in aging and chronic activation of the immune system reflects the accumulation of effector/senescent T cells. *Mech Ageing Dev* 2000;**121**:77–88.
- d'Angeac AD, Monier S, Pilling D, *et al*. CD57⁺ T lymphocytes are derived from CD57[−] precursors by differentiation occurring in late immune responses. *Eur J Immunol* 1994;**24**:1503–11.
- Palmer BE, Blyveis N, Fontenot AP, *et al*. Functional and phenotypic characterization of CD57⁺CD4⁺ T cells and their association with HIV-1-induced T cell dysfunction. *J Immunol* 2005;**175**:8415–23.
- Jimenez-Martinez MC, Linares M, Baez R, *et al*. Intracellular expression of interleukin-4 and interferon-gamma by a *Mycobacterium tuberculosis* antigen-

- stimulated CD4⁺ CD57⁺ T-cell subpopulation with memory phenotype in tuberculosis patients. *Immunology* 2004;**111**:100–6.
- 8 **Okada T**, Iiai T, Kawachi Y, *et al*. Origin of CD57⁺ T cells which increase at tumour sites in patients with colorectal cancer. *Clin Exp Immunol* 1995;**102**:159–66.
 - 9 **Maeda T**, Yamada H, Nagamine R, *et al*. Involvement of CD4⁺, CD57⁺ T cells in the disease activity of rheumatoid arthritis. *Arthritis Rheum* 2002;**46**:379–84.
 - 10 **Tan LC**, Mowat AG, Fazou C, *et al*. Specificity of T cells in synovial fluid: high frequencies of CD8⁺ T cells that are specific for certain viral epitopes. *Arthritis Res* 2000;**2**:154–64.
 - 11 **Jabs DA**, Nussenblatt RB, Rosenbaum JT; Standardization of Uveitis Nomenclature (SUN) Working Group. Standardization of uveitis nomenclature for reporting clinical data. Results of the First International Workshop. *Am J Ophthalmol* 2005;**140**:509–16.
 - 12 **Sallusto F**, Geginat J, Lanzavecchia A. Central memory and effector memory T cell subsets: function, generation, and maintenance. *Annu Rev Immunol* 2004;**22**:745–63.
 - 13 **Sallusto F**, Lenig D, Forster R, *et al*. Two subsets of memory T lymphocytes with distinct homing potentials and effector functions. *Nature* 1999;**401**:708–12.
 - 14 **Fritsch RD**, Shen X, Sims GP, *et al*. Stepwise differentiation of CD4 memory T cells defined by expression of CCR7 and CD27. *J Immunol* 2005;**175**:6489–97.
 - 15 **Tomiyama H**, Takata H, Matsuda T, *et al*. Phenotypic classification of human CD8⁺ T cells reflecting their function: inverse correlation between quantitative expression of CD27 and cytotoxic effector function. *Eur J Immunol* 2004;**34**:999–1010.
 - 16 **Faint JM**, Annels NE, Curnow SJ, *et al*. Memory T cells constitute a subset of the human CD8⁺CD45RA⁺ pool with distinct phenotypic and migratory characteristics. *J Immunol* 2001;**167**:212–20.
 - 17 **Hamann D**, Kostense S, Wolthers KC, *et al*. Evidence that human CD8⁺CD45RA⁺CD27⁺ cells are induced by antigen and evolve through extensive rounds of division. *Int Immunol*, **11**:1027–33.
 - 18 **Guenane H**, Hartani D, Chachoua L, *et al*. Production of Th1/Th2 cytokines and nitric oxide in Behçet's uveitis and idiopathic uveitis. *J Fr Ophthalmol* 2006;**29**:146–52.
 - 19 **Takata H**, Takiguchi M. Three memory subsets of human CD8⁺ T cells differently expressing three cytolytic effector molecules. *J Immunol* 2006;**177**:4330–40.
 - 20 **Kaech SM**, Hemby S, Kersh E, *et al*. Molecular and functional profiling of memory CD8 T cell differentiation. *Cell* 2002;**111**:837–51.
 - 21 **Sada-Ovalle I**, Torre-Bouscoulet L, Valdez-Vazquez R, *et al*. Characterization of a cytotoxic CD57⁺ T cell subset from patients with pulmonary tuberculosis. *Clin Immunol* 2006;**121**:314–23.
 - 22 **Hingorani R**, Monteiro J, Furie R, *et al*. Oligoclonality of V beta 3 TCR chains in the CD8⁺ T cell population of rheumatoid arthritis patients. *J Immunol* 1996;**156**:852–8.
 - 23 **Le Priol Y**, Puthier D, Lecureuil C, *et al*. High cytotoxic and specific migratory potencies of senescent CD8⁺CD57⁺ cells in HIV-infected and uninfected individuals. *J Immunol* 2006;**177**:5145–54.