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γδ T Cell Receptor–Bearing Lymphocytes during Epstein-Barr Virus Infection

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Lymphocytes bearing γδ T cell receptors (TCR) constitute a minor subpopulation of human peripheral blood lymphocytes. Their role and function during microbial infections are largely unknown. In 10 patients with Epstein-Barr virus–induced infectious mononucleosis, the γδ TCR–expressing T cell population expanded during the acute phase. These cells were largely δTCS1⁻, CD4⁻, and CD8⁻ but expressed activation antigens such as human leukocyte antigen–DR and CD38. The convalescent phase of infectious mononucleosis was characterized by a relative persistence of γδ T cells. Together these data suggest a possible role of γδ T cells in the control of primary Epstein-Barr virus infection in humans.

Two different forms of T cell receptors (TCR) have been identified in peripheral blood T lymphocytes: the αβ, expressed on most CD4 and CD8 cells, and the γδ heterodimer [1, 2]. The γδ TCR–expressing T cells represent only a minor population (1%–10%) of normal peripheral blood lymphocytes.

Monoclonal antibodies reacting with different epitopes of the γδ TCR chains have been raised [3]. TCRδ1 reacts with all γδ TCR–bearing T lymphocytes (pan–γδ TCR marker) [4], δTCS1 has a more restricted reactivity in peripheral blood lymphocytes [5], while BB3 reacts with the Vδ2-encoded region of the γδ TCR [6].

The antigenic profile of γδ T lymphocytes shows that usually these cells lack CD4 and CD8, while the expression of CD2, CD7, CD45R, and Leu8 is comparable to that of the CD3 αβ population [1]. Also similar to CD3 αβ cells, activation markers such as CD25 and human leukocyte antigen (HLA)-DR are absent on the γδ subset.

The distribution of γδ T cells in the mouse suggests that they dominate lymphocyte subpopulations in epithelia [7], although the situation in humans is less clear. Several experiments showed that γδ T cells have cytotoxic activity [7, 8], accounting for some of the non–major histocompatibility complex restricted cytotoxic activity seen in normal peripheral blood lymphocytes.

Infectious mononucleosis is a self-limiting lymphoproliferative disease caused by the Epstein-Barr virus (EBV). The resolution of this disease is primarily dependent on a cell-mediated immune response [9, 10]; both suppressor and non–HLA restricted cytotoxic T cells have been shown to develop in vivo during acute EBV infection and to be responsible for in vitro control of the EBV-infected target cell replication [9–11].

The transient nature of these alterations suggests that an immunoregulatory mechanism operates during acute infectious mononucleosis. Several modifications of lymphocyte subsets have been described in this phase of the disease, but the precise nature of this mechanism is still debated. We hypothesized that γδ T cells could play a role during acute EBV infection; our study describes the cytofluorographic analysis, by selected monoclonal antibody combinations, of peripheral blood T cells during the acute and convalescent phases of infectious mononucleosis.

Materials and Methods

Patients. Ten patients diagnosed clinically with infectious mononucleosis (aged 15–25) were studied. Clinical diagnosis was based on fever, lymphadenomegaly, splenomegaly, and lymphocytosis with atypical lymphocytes in the peripheral blood. All patients had a positive Paul-Bunnell-Davidsohn test and high titers of IgM

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Table 1. Lymphocyte marker analysis in acute and convalescent infectious mononucleosis patients.

Marker	Acute		Convalescent		Control	
	%	Cells/mm³	%	Cells/mm³	%	Cells/mm³
CD3	80 ± 7	5907 ± 2060*	76.2 ± 8	2040 ± 640	75.3 ± 6	1750 ± 380
CD4	19.6 ± 7†	1365 ± 520	32.2 ± 8	842 ± 249	42.5 ± 4	980 ± 240
CD8	47.8 ± 11†	3594 ± 1670*	29.6 ± 8	778 ± 297	27.9 ± 4	649 ± 195
CD8 CD38	35.2 ± 11*	2300 ± 845*	78.6 ± 6	193 ± 210	5.0 ± 3	114 ± 79
TCRδ1	8.9 ± 5	708 ± 600*	15.4 ± 6†	439 ± 240†	5.8 ± 3	134 ± 57
δTCS1	0.65 ± 0.5	50 ± 30	1.9 ± 2.2	43 ± 59	1.4 ± 0.5	35 ± 15
TCRδ1 DR	4.0 ± 3*	383 ± 150	ND	ND	0.1 ± 0.1	3 ± 1
TCRδ1 CD38	5.2 ± 4*	515 ± 175*	ND	ND	0.1 ± 0.1	2 ± 0.9
TCRδ1 CD8	0.17 ± 0.1	12 ± 10	ND	ND	0.8 ± 0.1	17 ± 2

NOTE. Results are expressed as mean ± SD. ND = not determined.
* $P < .01$ compared with control.
† $P < .05$ compared with control.

antibodies to viral capsid antigen (VCA) of EBV. Peripheral blood was drawn in the acute phase and after 4 weeks (convalescent phase).

The control population consisted of normal healthy volunteers of the same age group. The control samples were obtained at the same time as the acute infectious mononucleosis samples.

Leukocyte isolation. Peripheral blood was incubated with Plasmagel for 30 min at 37°C. The leukocyte-rich buffy coat was recovered and, after lysis of the remaining red cells with an NH_4Cl solution, resuspended in phosphate-buffered saline (PBS).

Monoclonal antibodies. Monoclonal antibodies OKT3 (CD3), OKT4 (CD4), OKT8 (CD8) (Ortho D.S., Milan, Italy), Leu17 (CD38), anti-HLA-DR (Becton Dickinson, Milan, Italy), TCRδ1 (pan-γδ T cell marker), and δTCS1 (recognizing a subset of γδ T cells) (T Cell Science Medical System, Genoa, Italy) were used at appropriate dilutions. Leukocytes (~1 × 10^6 from buffy coat) were incubated with the FITC-labeled antibody for 30 min at 4°C. The cells were then washed with PBS. Dual immunofluorescence was done by serial incubations with FITC TCRδ1 or FITC OKT8 and phycoerythrin (PE) HLA-DR, Leu17, OKT8, or OKT4.

Cytofluorographic analysis. Cytofluorographic analysis was performed on a FACS 440 equipped with a Consort 40 computer. Background fluorescence was determined with fluorochrome-labeled mouse immunoglobulins. Dual immunofluorescence was calculated by computer-assisted analysis, after electronic subtraction of the signals, to obtain true FITC and PE outputs in the respective detectors.

Lymphocyte counts were obtained by standard hemocytometric counters. IgM and IgG anti-VCA were obtained by standard immunofluorescence assay on HR 1K cells [12] (Gull Laboratories, Salt Lake City). Statistical analysis was performed using Student's *t* test.

Results

The lymphocytosis associated with acute infectious mononucleosis was shown to be the result of heterogeneous expansion of lymphocyte subpopulations. During the acute phase, the absolute number of CD3 T cells was significantly elevated ($P < .01$) compared with that in healthy controls (table 1). Despite the decreased percentage of CD4 cells, their absolute number was increased. A fivefold increase of absolute CD8 cell numbers, with most bearing the activation marker CD38, was the most dramatic event during acute infectious mononucleosis.

The TCRδ1 monoclonal antibody, a pan-γδ T cell marker, recognized 8.9% ± 5% of total lymphocytes (708 ± 600 cells/mm³, $P < .01$ compared with healthy controls). Dual-parameter immunofluorescence analysis demonstrated that 4.0% ± 3% (383 ± 150) and 5.2% ± 4% (515 ± 175) of the cells coexpressed TCRδ1 and, respectively, HLA-DR or CD38. Both results were highly significant compared with healthy controls ($P < .01$, table 1). Only 0.17 ± 0.1% of the cells were $TCR\delta1^+$ and $CD8^+$, while no TCRδ1 and CD4 coexpression was found.

Illustrated in figure 1 are representative cytofluorographs of an acute infectious mononucleosis patient and a normal control analyzed by dual immunofluorescence with TCRδ1 and anti-HLA-DR or anti-CD38 combinations. We observed a significant correlation between the extent of lymphocytosis and the absolute number of γδ T cells: The correlation coefficient between the absolute number of total $CD3^+$ and $TCR\delta1^+$ lymphocytes was 0.71 ($P < .01$). No relationship between TCRδ1 cell numbers and IgG VCA antibody titers was found. The δTCS1 antibody stained 0.65% ± 0.5% (50 ± 30 cells/mm³), not different from normal controls.

The convalescent period of infectious mononucleosis was characterized by a normalization of lymphocyte absolute numbers and of CD4 and CD8 subsets and by an almost complete disappearance of activation markers (table 1). At this time the TCRδ1 T lymphocytes constituted 15.4% ± 6% (439 ± 260 cells/mm³, $P < .05$ vs. normal controls). These data indicate that during convalescence the percentage of γδ T cells increases while their absolute number remains elevated.

Discussion

The $CD3^+$, γδ TCR^+ T cell subset recently has been characterized in the peripheral blood of normal adults [1]. Little

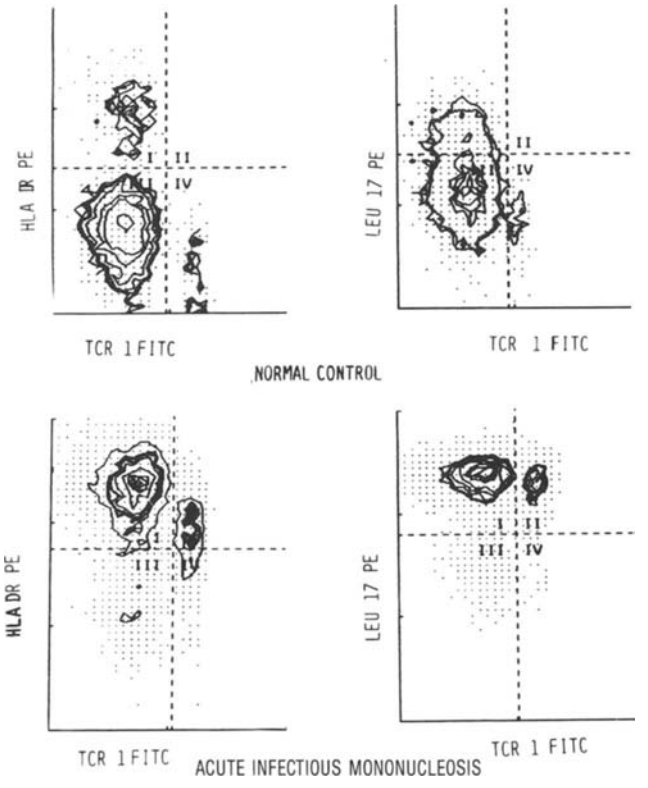

Figure 1. Coexpression of TCRδ1 with HLA-DR and Leu17 (CD38) in peripheral blood from an acute infectious mononucleosis patient and a normal control. The contour plots were divided into four quadrants: (I) cells with phycoerythrin (PE) fluorescence only; (II) cells with FITC and PE fluorescence; (III) unstained cells; and (IV) cells with FITC fluorescence only.

is presently known about the functions of this subset in vivo and its variations in microbial infections. Recently it has been demonstrated that γδ T cells can be activated in vivo after infection with *Mycobacterium tuberculosis*, indicating that this subset may have a role in generating a primary immune response to microorganisms [13, 14]. An intriguing hypothesis is that γδ T cells may have T cell receptors recognizing proteins homologous to various microorganisms, such as heat shock proteins [13].

The TCRδ1 lymphocytes have been shown to be normal during human immunodeficiency virus (HIV) infection; these data may be relevant to establish the HIV susceptibility of this subset [15] but are inconclusive regarding its role in the control of virus replication.

We therefore performed a cytofluorographic analysis of γδ T cell subsets during acute and convalescent phases of EBV-induced infectious mononucleosis. The γδ TCR subset, recognized by the pan-γδ TCRδ1 reagent, was increased during both the acute and convalescent phases; these cells were mainly $CD4^-$, $CD8^-$ but coexpressed HLA-DR and CD38, indicating a significant degree of activation. The δTCS1 fraction was not modified during the disease course. The expansion of the γδ T cell subset was correlated with the absolute number of total $CD3^+$ lymphocytes during the acute phase, while during convalescence we showed a relative persistence of γδ T cells, accounting for 15.4% of total peripheral blood lymphocytes.

The immunoregulatory mechanism operating during acute EBV infection is not completely understood; it has been demonstrated to be partially dependent on suppressor and non-HLA restricted cytotoxic T cells. Available information suggests that suppressor and cytotoxic fractions represent different mechanisms of host defense in patients acutely infected with EBV. Suppressor cells seem to limit the disease by inhibiting lymphocyte activation and proliferation [9]; by contrast cytotoxic T cells would eliminate EBV-infected cells that are not susceptible to inhibition by the suppressor mechanism [9].

Since γδ T cells function mainly as non-HLA restricted cytotoxic cells [7], we suggest that the expansion and activation of this subset during acute EBV infection may be a relevant immunoregulatory mechanism. The behavior of the γδ T cell subset during other herpesvirus infections remains to be established. Our preliminary data on patients with acute varicella show normal TCRδ1 values.

In conclusion, our data demonstrate that the expanded lymphocyte population in acute infectious mononucleosis is heterogeneous. Although $CD8^+$, $CD38^+$ cells were predominant, the demonstration of an expanded and activated $CD3^+$, γδ TCR^+ population suggests that this subset may contribute to the immunoregulatory mechanism during EBV infection. Further work is necessary to elucidate the importance of γδ TCR-expressing T cells in the control of viral diseases.

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Abnormalities of T Cell Immunoregulation in Hemorrhagic Fever with Renal Syndrome

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The functions of spontaneous suppressor T cells and T lymphocyte subsets in patients with hemorrhagic fever with renal syndrome were compared. In the early stages of disease, decreased activity of spontaneous suppressor T cells was concurrent with increased numbers of $CD8^+$ cells and a reversed CD4:CD8 ratio. These changes were related to abnormalities in serum C3 level and circulating immune complexes. In the recovery stages of the illness, spontaneous suppressor T cell activity and T cell subsets returned to normal levels.

Hemorrhagic fever with renal syndrome (HFRS) is a severe infectious disease with high mortality in the People's Republic of China [1]. The pathogenesis of the disease remains unclear; however, the absence of clear evidence for virus cytopathology in fatal cases of HFRS and the coincidence of the humoral immune response with disease onset have led to speculation regarding an immunopathologic basis for this disease [2–5]. Accumulated data demonstrate that there are some obvious disturbances of host immune functions during the course of HFRS, such as activation of complement in vivo [2]; low reactivity of lymphocytes to mitogens [3]; the formation of circulating immune complex; deposits of immune complexes in glomerular capillary basement membranes, mesangium, and tubular basement membranes [3, 4]; and elevated specific IgE in serum from patients with HFRS [5]. Little is known, however, about the relation between these immunologic disturbances and the functions of regulatory T lymphocytes in HFRS.

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The present study was undertaken to investigate simultaneously activity of spontaneous T suppressor lymphocytes (STsL) and T cell subsets in patients with HFRS, in an attempt to understand immunoregulation in HFRS.

Materials and Methods

Patients and controls. The subjects of the STsL function assay were 83 cases of HFRS and 23 healthy donors who served as controls. The subjects of the study of T cell subsets were 64 patients with HFRS, 25 of whom simultaneously underwent the STsL function test; 17 healthy persons served as controls.

All controls were age- and gender-matched. The cases of HFRS were diagnosed clinically and confirmed serologically using the immunofluorescent antibody (IFA) method by detecting a rise in titer of antibody to Hantaan virus. No patients were on immunotherapy during the study.

Reagents. Reagents and suppliers were mitomycin C (Sigma Chemical, St. Louis); concanavalin A (ConA; Vector Laboratories Burlingame, CA); [^{3}H]thymidine (Institute of Atomic Energy Research, Beijing); and anti-human T lymphocytes (CD3, CD4, and CD8), monoclonal antibodies (mAbs), and FITC-labeled rabbit antibodies to mouse IgG (Institute of Biological Products, Wuhan, People's Republic of China).

STsL function assay. STsL activity was measured using the method described by Gattringer et al. [6], with slight modification. Briefly, freshly isolated peripheral blood monocytes (PBM), prepared from