Coexpression of Fcγ Receptor IIIA and Interleukin-2 Receptor β Chain by a Subset of Human CD3+/CD8+/CD11b+ Lymphocytes

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In this study we identify and characterize a subset of human peripheral blood T cells, present in all individuals, that has features previously described for T cells either separately or in special circumstances. These cells are found in purified suspensions of resting peripheral blood lymphocytes within the CD8+ T lymphocytes, express αβ T cell receptor (TCR), and can be identified and isolated because of high-density expression of surface CD11b (TCRαβ+/CD3+/CD8+/CD11b+ cells). They coexpress constitutively the IL-2 receptor β chain, FcγRIIIA, and CD56. Although they do not mediate spontaneous cytotoxicity, CD3+/CD8+/CD11b+ cells have cytotoxic potential, demonstrated in redirected cytotoxicity assays with P815 target cells in the presence of anti-FcγRIII (CD16) or anti-CD3 monoclonal antibodies. Stimulation of CD3+/CD8+/CD11b+ cells with rIL-2 induces proliferation, cytotoxicity against NK-sensitive and NK-resistant target cells, and expression of surface activation antigens, including IL-2 receptor α chain (CD25). CD3+/CD8+/CD16+ cells clones with cytotoxic functions including those mediated by engagement of surface CD16 were obtained by limiting-dilution cloning of purified CD3+/CD8+/CD11b+ cells in the presence of rIL-2 and autologous feeder cells. Our data support the hypothesis that the CD3+/CD8+/CD11b+/CD16+ cells represent a discrete peripheral blood lymphocyte subset that could be the physiological counterpart of that expanded in several pathological conditions and in large granular lymphocyte lymphocytosis.

INTRODUCTION

In humans, T cells of the CD8+ subset respond to recombinant interleukin-2 (rIL-2) in vitro in the absence of antigenic or mitogenic stimulation (1-5). Several reports suggest that this depends on the constitutive expression of the β chain (p75; intermediate-affinity receptor) but not of the α chain (p55) of the IL-2 receptor (IL-2R) by a number of CD8+ cells (6-12). However, it is unclear whether or not IL-2Rβ chain is expressed exclusively on a specialized subpopulation of CD8+ T cells. The low proportion of CD8+ cells with intermediate-affinity IL-2R, together with the presence of some activated cells with high-affinity IL-2R (α+β chain), has posed considerable challenge to further investigation (2, 5, 10-13).

Previously, we described a subpopulation of CD3+/CD8+ T cells with large granular lymphocyte (LGL) morphology that, upon stimulation with rIL-2, proliferates and generates MHC-nonrestricted cytotoxic cells (14). These CD8+ T cells did not express detectable IL-2R α chain and were included in a relatively small fraction of cells coexpressing CD11b.

In the present study, we demonstrate that cells of the CD3+/CD8+/CD11b+ subset express constitutively not only the IL-2Rβ chain, but also CD56 and the transmembrane form of FcγRIII (FcγRIIIA), which is functional in triggering cell-mediated cytotoxicity. This cell subset was present in all individuals tested. Moreover, in suspensions of CD8+ cells depleted of activated cells, the CD3+/CD8+/

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CD11b+ cells were the only ones capable of responding to rIL-2 and developing MHC-nonrestricted cytotoxicity. These observations have been confirmed in experiments at the clonal level.

Although cells with some of the above properties have been described within T cells from selected individuals (15), the present study provides criteria to identify unequivocally, and to separate, a distinct subset of CD8+ T lymphocytes from the peripheral blood of most healthy subjects. The phenotypic and functional features of the CD3+/CD8+/CD11b+ peripheral blood lymphocytes (PBL) resemble those of the T cells found expanded in the majority of cases of the so called LGL lymphocytosis (16, 17); the possibility that these cells represent the normal counterpart of the lymphocytes that give origin to the LGL lymphocytosis is discussed.

MATERIALS AND METHODS

Cell Preparations

Peripheral blood mononuclear cells (PBMC) were isolated from buffy coats of healthy individuals by centrifugation on Ficoll–Hypaque (FH) density gradients (18). Cell preparations were depleted of monocytes by adherence to plastics (45 min, 37°C).

Monoclonal Antibodies and Immunofluorescence

The monoclonal antibodies (mAb) used were: anti-CD3, OKT3 (IgG2a) (Ortho Diagnostics, Raritan, NY), and F23.1 (IgG1), kindly provided by Dr. A. Lanzavecchia (Basel Institute for Immunology, Switzerland); anti-CD4, OKT4 (IgG2b), anti-CD8, OKT8 (IgG2a) (Ortho Diagnostics); anti-CD56, NKH1-A (IgM) (Coulter Corporation, Hialeah, FL), and Leu19 (IgG1) (Becton Dickinson, San José, CA); anti-CD16, Leu11b (IgM) (Becton Dickinson), B73.1 (IgG1) from one of us (B.P.) (19), 3G8 (IgG1) obtained from cells kindly provided by Dr. J. Unkeless (Mount Sinai Medical School, New York), and 1D3 (IgG1) kindly provided by Dr. J. Griffin (Dana Farber Cancer Institute, Boston, MA); anti-HLA-DR, OKDR (IgG2a) (Ortho Diagnostics); anti-CD69, MLR3 (IgG2a) kindly donated by Dr. M. E. Cosulich (IST, Genova, Italy) (20); anti-CD71, Tfr (IgG2a) (Becton Dickinson); anti-TCR αβ, WT31 (IgG1) (Becton Dickinson); anti-TCR γδ, BB3 (IgG1), provided by Dr. A. Moretta, (University of Genova, Italy) (21), and δ TCS-1 (IgG 1) (T Cell Science, Boston, MA); anti-CD20, B1 (IgG2a) (Coulter Corporation); anti-CD14, MO2 (IgM) (Coulter Corporation); anti-CD11b, MO1 (IgM) (Coulter Corporation), OKM1 (IgG2b) (Ortho Diagnostics), and CR3 (IgG2a) (Becton Dickinson). Antibodies directed against the p75 (β-chain) and the p55 (CD25; α-chain) subunits of the IL-2R were Tu27 (IgG1), a gift from Dr. K. Sugamura (Tohoku University, Sendai, Japan) (22) and IL-2R (IgG1) (Becton Dickinson), respectively.

Indirect immunofluorescence was performed with fluorescein isothiocyanate (FITC)- or phycoerythrin (PE)-conjugated goat anti-mouse (GAM) Ig isotype antibodies (Southern Biotechnologies, Birmingham, AL) as second anti-Ig reagents. For two-color immunofluorescence, mAb of different isotypes and the corresponding FITC- or PE-conjugated GAM Ig isotypes were used. The samples were analyzed using a FACStar cytometer (Becton Dickinson Immunocytometry Systems, San José, CA). The intensity of fluorescence (FI) was expressed on a log scale. Cells incubated with isotype-matched irrelevant mouse monoclonal Ig and the second reagents were used as negative controls. Single- and two-color stainings of cells previously fractionated using mAb and indirect antiglobulin rosetting was performed on cells cultured overnight and washed several times with RPMI 1640. This procedure allowed removal of the mAb from the cell surface, as confirmed by indirect immunofluorescence with FITC-conjugated GAM Ig antisera.

Cell Fractionation Procedures

T cell populations were purified from PBL after rosetting with sheep erythrocytes (E) at 29°C (low-affinity rosettes) (23) (CD3+ PBL) and separation from the nonrosetting cells (B, NK cells) on FH. To obtain homogeneous populations of CD8+ T lymphocytes, CD3+ cells were incubated with a mixture of anti-CD4, anti-CD20, and anti-CD14 mAb and rabbit serum as a source of complement (C) (Cederlaine, Hornby, Ontario, CA) (24). Live cells were obtained after FH density gradients. Alternatively, mAb and indirect antiglobulin rosetting using GAM Ig (Southern Biotechnologies) antiserum-coated ox E were used (24, 25). Rosetting (Ab+) and nonrosetting (Ab−) cells were separated on FH density gradients.

The CD3+/CD8+ cells were separated into CD11b+ and CD11b− cells by indirect antiglobulin