Brief Definitive Report

LYMPHOKINE REGULATION OF CD45R EXPRESSION ON HUMAN T CELL CLONES

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The T-200 (CD45R) family of molecules are expressed with selective isoforms through a process of exon splicing on functionally distinct T cell subsets (1–3). Expression of the higher molecular weight (200,000–220,000 M_r) isoforms on the surface of resting $CD4^+$ T cells has been associated with downregulation of immune responses (4–7), which may relate to the tyrosine phosphatase activity of the CD45R complex (8–11). Whether the expression of the higher molecular weight isoforms recognized by anti-CD45RA (2H4) mAbs represents different lineages of T cells and/or a stage in the sequential pathway of T cell differentiation remains unknown (4–7). The decreased expression of CD45RA with T cell activation of bulk T cell populations and the inability of investigators to identify a stable human T cell clone expressing cell surface CD45RA molecules has been evidence suggesting that CD45RA expression may represent a marker of naive T cells, while expression of the lower molecular weight isoforms of the T-200 complex recognized by anti-UCHL-1 mAb represents memory T cells (5–7).

We now report the characterization of a smaller subpopulation of T cell clones that were induced to express the CD45RA molecule with activation by ionomycin. This CD45RA expression was also upregulated in a specific manner by the cytokines IL-1 and IL-6. These results indicate CD45RA expression can define T cell lineages in a subpopulation of activated T cells.

Materials and Methods

Cloning. PBMC were directly cloned at less than one cell per well with 10^5 autologous, irradiated (5,000 rad) PBMC and purified PHA (PHA.P) (1.0 μg/ml) (Wellcome Diagnostics, Beckenham, UK) in 96-well V-bottomed plates in standard media consisting of 10% pooled human AB serum in RPMI (Whitaker M. A. Bioproducts, Walkersville, MD), 2% glutamine (Gibco Laboratories, Grand Island, NY), and 1% penicillin/streptomycin (Gibco Laboratories). 48 h later, 0.1 ml of media containing 10% IL-2 (ABI, Columbia, MD) and 50 U/ml of rIL-4 (Genzyme, Boston, MA) was added to each well. Cultures were fed with IL-2 and IL-4 every 3–5 d until approximately day 12, when all the wells were passed to 96-well U-bot-

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tomed plates. Growth-positive wells were scored macroscopically and transferred into V-bottomed plates with 5,000 cloned T cells per well with 10^5 allogeneic, irradiated (5,000 rad) mononuclear cells and PHA.P with IL-2 plus IL-4. When there were 2–10 × 10^4 T cells per V-bottomed well (usually 3–5 d), clones were transferred to flasks. T cell clones were restimulated every 10–14 d, as above.

Resting T cell clones (stimulated 7 d earlier with PHA.P and feeder cells) were activated with ionomycin (Calbiochem-Behring Corp., La Jolla, CA) (100 ng/ml) and PMA (Sigma Chemical Co., St. Louis, MO) (1 ng/ml) and incubated for 3 or 12 h. Cytofluorographic analysis after staining of resting and activated clones was performed by means of either direct immunofluorescence with phycoerythrin (PE)-conjugated anti-CD45RA$^+$ (2H4) mAb (Coulter Immunology, Hialeah, FL) with background fluorescent reactivity determined using PE-conjugated mouse Ig of the same isotype as a control, or in some experiments, by indirect immunofluorescence (4). Flow cytometric analysis was performed using an Epics C flow cytometer (Coulter Electronics, Hialeah, FL). All incubations were for 30 min with two washes between steps and before analysis.

Immunoprecipitation. T cells were labeled by lactoperoxidase-catalyzed iodination, (1) washed, and solubilized in lysis buffer (1% NP-40 [wt/vol] in 20 ml Tris-HCL buffer, pH 8.0, containing 100 mM NaCl and 1 mM PMSF). The lysate was then incubated with 5 μl of the mAb 9.4 (a kind gift of Dr. P. Martin, Seattle, WA) before precipitating with 50 μl of 10% (wt/vol) protein A-Sepharose (12). Immunoprecipitates were washed three times in lysis buffer before analysis on a 5–7% gradient gel.

Cytokines. T cell clones were purified by density centrifugation on a Ficoll/Hypaque gradient after being stimulated between 7 and 14 d earlier with PHA.P and accessory cells irradiated with 5,000 rad. This population consists of only T cells with no viable accessory cells. 10^6 T cell clones were incubated with complete media for 18 h at 37°C with IL-1 (3 U/ml; Genzyme), rIL-2 (10 U/ml), rIL-4 (10 U/ml; Genzyme), rIL-5 (1 U/ml), rIL-6 (10 U/ml), and IFN-γ (1,000 U/ml; Genzyme) (rIL-2, rIL-5, and rIL-6 were gifts from Drs. Steven Clark and Ken Jacobs, Genetics Institute, Cambridge, MA), and stained with anti-2H4 mAb and mouse Ig as described above.

Results

A total of 81 T cell clones were derived from two subjects for examination of CD45RA expression. In subject Ar, 7/41 T cell clones expressed CD45RA with activation. Of these, 4/9 (44%) were CD8$^+$, and 3/32 (9%) were CD4$^+$. Another series of T cell clones were generated from subject Re; of which, two of four CD8$^+$ clones expressed CD45RA with activation while none of the CD4$^+$ clones were positive (Fig. 1). All of the clones expressed high densities of cell surface CD45RO (UCHL-1) and CDw29 (4B4) molecules both at rest and with activation.

A number of T cell clones expressing CD45RA from subject Re were expanded to large numbers to further investigate CD45RA regulation. The kinetics of the CD45RA expression was examined by activating clone Re.F with ionomycin and PMA. Re.F expressed high densities of cell surface CD45RA within 12 h after activation, which was not observed among the other T cell clones (shown is clone Re.I, Fig. 1). Similarly, CD45RA expression in clone Re.F could be induced by ionomycin alone without PMA. In some experiments, CD45RA expression persisted throughout the cell cycle without further augmentation by reactivation, though it was most prominent in the T cell population with the greater forward angle and 90° light scatter further indicating that activated cells were preferentially induced to express the CD45RA$^+$ complex. These clones were carried in culture for ~11 mo before losing expression of the CD45RA molecule.

The specific isoforms expressed on the cell surface can be identified by immuno-

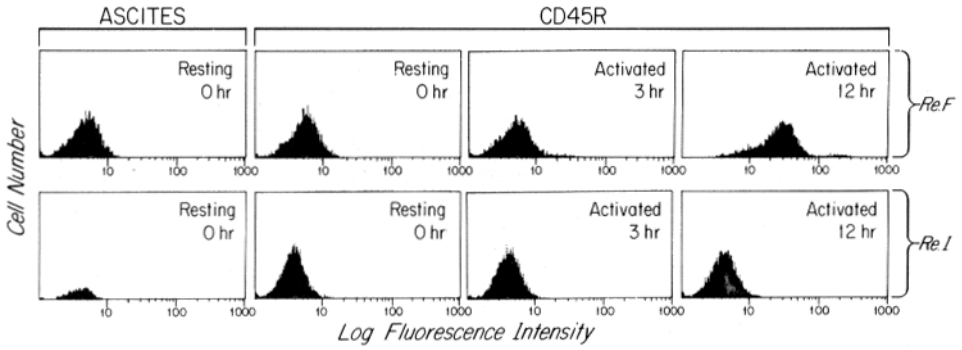

FIGURE 1. Activation of clone Re.F induces the expression of CD45RA (2H4) molecule. Clone Re.F and a representative clone, Re.I, were analyzed for the expression of CD45RA$^+$ (2H4) at rest or after stimulation with ionomycin and PMA for varying lengths of times. Cell numbers are represented on the vertical axis, and fluorescence, on a logarithmic scale, is on the horizontal axis. Background staining with ascites did not change after activation.

precipitation using anti-CD45R mAbs (1). As shown in Fig. 2, an additional band at 200,000 M_r was seen in activated clone Re.F compared with other anti-2H4$^-$ T cell clones corresponding to the specific isoform of CD45RA$^+$, confirming the phenotypic characterization. Expression of the 200,000 M_r isoform on the surface of clone Re.F appeared identical to that observed in resting peripheral blood T cells. There was no expression of the highest (220,000 M_r) molecular weight isoform of the T-200 complex on clone Re.F.

As activation by ionomycin alone could induce CD45RA$^+$ expression, the higher molecular weight isoform expression recognized by anti-2H4 mAb may have been induced by the autocrine release of cytokines. This was directly investigated by culturing the resting clones Re.F and RE.9, 7 d after last stimulation, with either IL-1, IL-2, IL-4, IL-5, IL-6, or IFN-γ for 18 h. It was found that IL-1 and IL-6 directly upregulated CD45RA$^+$ expression, while the other cytokines did not (shown is Re.F in Fig. 3). Similarly, five of the seven clones from subject Ar that could express CD45RA$^+$ were induced to express CD45RA after incubation with IL-1 (data not shown). This demonstrates that IL-1 induction of CD45RA expression is a property of a significant subset of human T cell clones.

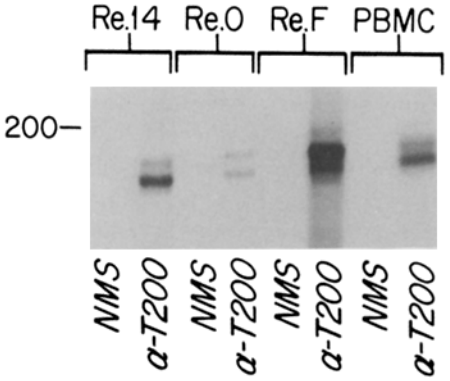

FIGURE 2. Phenotypic expression of CD45RA (2H4) molecule on Re.F correlates with 200,000 M_r isoform of T-200 complex. Three T cell clones and PBMC, activated as described in Fig. 1 for 12 h, were labeled by lactoperoxidase-catalyzed iodination and lysed in NP-40 lysis buffer for immunoprecipitation as previously described (1). Each sample was precipitated with either normal mouse serum or anti-T-200.

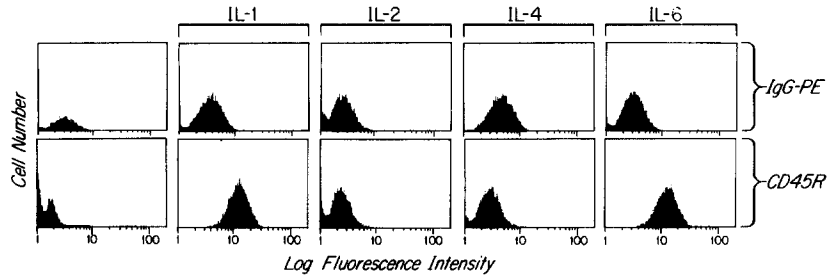

FIGURE 3. Incubation of clone Re.F with IL-1 or IL-6 induces CD45RA (2H4) expression. Clone Re.F, 7–10 d after stimulation, was incubated with the lymphokines as indicated for 18 h at 37°C and stained with anti-2H4 mAb as described in Fig. 1. rIL-5 and IFN-γ had no effect on CD45RA expression and are not shown. Cell numbers are represented on the vertical axis, and fluorescence, on a logarithmic scale, is on the horizontal axis.

TCR genes were examined on genomic DNA to examine whether the CD45RA$^+$ clones expressed TCR-α/β or TCR-γ/δ. Southern blot analysis showed a normally rearranged β chain of the TCR, indicating a TCR-α/β heterodimer (data not shown). The presence of a single rearrangement on Southern analysis also indicated the absence of contamination by second clones.

Discussion

T cells can be cloned directly from the peripheral blood before other in vitro manipulation with very high cloning efficiency using PHA and growth factors, including IL-2 and IL-4. Using this method, a panel of T cell clones could be derived that were representative of circulating T cells without regard to preselection by function or by kinetics of cell growth. This allowed us to identify a smaller population of T cells that could be induced to express the higher molecular weight isoforms of the T-200 complex, CD45RA, recognized by the anti-2H4 mAb. These studies indicate that CD45RA (2H4) expression can define a T cell lineage in a subpopulation of T cells that can at times be regulated by IL-1 and IL-6.

Recent evidence has indicated that the CD45 T-200 complex expresses tyrosine phosphatase activity (8). In addition, CD4 and CD8 molecules are known to associate with p56^{lck} protein kinase (9, 10), which can phosphorylate members of the CD3 complex (13). CD45 is thought to function in the regulation of immune responses by dephosphorylating tyrosine residues in target proteins, such as the ζ chain of the CD3/TCR complex (11, 14). It may be speculated that the ability of IL-1, IL-6, or ionomycin to induce CD45RA expression is linked to tyrosine phosphatase activity, which then impinges on CD4/Ti/CD3 interactions. Further studies of tyrosine phosphatase activity induced by IL-1 or IL-6 signaling in relationship to functional suppression are presently under investigation.

Clear parallels have been demonstrated between the activities of IL-1 and IL-6, potentially explaining why both cytokines could regulate CD45RA expression in the present investigation. It is also possible that IL-1 or ionomycin induced CD45RA expression indirectly by autocrine secretion of IL-6, as IL-1 is a potent inducer of IL-6 secretion in many cell types (15). This possibility is consistent with the presence

of inducible IL-6 mRNA in clone Re.F (data not shown), though the lack of inducible IL-1 mRNA indicates that the effect of IL-6 is not mediated through IL-1 secretion.

Previous studies by some laboratories examining bulk T cell populations suggested that expression of the CD45RA molecule disappears with T cell activation (5, 6). In the present studies, direct single cell cloning has allowed for a more detailed analysis of less frequent cell populations. Moreover, recent work by Rothstein et al. (16) has shown that 30–50% of $2H4^+$ T cells stimulated with Con A express the CD45RA isoforms in a stable fashion (16). These $CD45RA^+$ cells remain functionally distinct as suppressor cells, in contrast to $CD45RA^-$ cells, which provide increased levels of help. Nevertheless, these studies in total do not exclude the possibility that CD45RA expression may also represent a sequential differentiation pathway in certain T cell subsets.

Taken together, these investigations indicate that CD45RA (2H4) expression can be observed on a subpopulation of long-term T cell clones that can at times be regulated by the cytokines IL-1 and IL-6. Moreover, these results may have implications regarding the regulatory actions of IL-1 and IL-6, with their ability to increase CD45RA expression in subpopulations of human T cells.

Summary

Whether the expression of higher molecular weight isoforms of the T-200 complex represents different lineages of T cells and/or a sequential stage of the differential pathway of T cells has been unclear. Understanding T cell expression of higher molecular weight isoforms of the T-200 complex (CD45R) may be important because of their association with regulation of immune responses. By direct single cell cloning, we observed a number of long-term T cell clones that expressed CD45RA (2H4). CD45RA expression could be further regulated by ionomycin or the cytokines IL-1 and IL-6, but not IL-2, IL-4, or IFN-γ. These results indicate that CD45RA expression may define T cell lineages of activated T cells partially controlled by the cytokines IL-1 and IL-6. Further, these results may associate regulatory actions of IL-1 and IL-6 with their ability to increase CD45RA expression in subpopulations of human T cells.

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