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Special Article

Isoforms of the CD45 Common Leukocyte Antigen Family: Markers for Human T-Cell Differentiation

LORAN T. CLEMENT[1]

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The diverse host defense and immunoregulatory functions of human T cells are performed by phenotypically heterogeneous subpopulations. Among the membrane antigens that are differentially expressed by reciprocal human T-cell subsets are the CD45RA and CD45RO isoforms of the common leukocyte antigen family, which have been hypothesized to identify "naive" and "memory" T cells, respectively. The CD45RA antigen is first expressed by T-lineage cells relatively late during their intrathymic maturation and continues to be expressed by most T cells in the immunologically naive neonate. With increasing age and antigenic exposure, however, CD45RA−/RO+ cells become more prevalent in the circulation and comprise the majority of cells in tissues. Analyses of the functional capabilities of CD4+CD45RA+ and CD4+CD45RO+ cells have shown that proliferative responses to "memory" recall antigens or the ability to provide help for antibody production are functions uniquely performed by CD4+CD45RA−/RO+ cells. The major immunoregulatory functions described for CD4+CD45RA+ cells involve suppression of immune responses, either directly or via the induction of suppressor activity by CD8+ cells. Two general models of differentiation have been proposed to describe the lineal relationship of these T-cell subsets. Although these subsets could represent mature, phenotypically and functionally stable progeny arising from separate differentiation pathways, there is considerable experimental support for the hypothesis that CD45RA−/RO+ cells are "memory" cells that derive from "naive" or "virgin" CD45RA+/RO− precursors via an activation-dependent postthymic differentiation pathway. Altered frequencies of CD45RA+ and CD45RO+ T cells have been observed in a variety of different clinical conditions, particularly diseases manifesting altered immune function. These findings have contributed new information concerning the physiological events regulating the *in vivo* generation of these T-cell subsets. In addition, they may provide clues to the pathogenetic processes associated with certain diseases.

KEY WORDS: Lymphocyte differentiation; T-cell subpopulations; CD45 isoforms; common leukocyte antigens.

INTRODUCTION

The efficient functioning of the immune system is dependent on the orderly differentiation of functionally distinct lymphocyte subpopulations from a common hematopoietic stem cell. During this process, lymphoid cells acquire the ability to distinguish self from nonself and to respond adaptively to foreign antigenic stimuli. In addition, certain lymphocyte subpopulations acquire functional capabilities that contribute to host defense mechanisms, while other subsets develop immunoregulatory functions that govern the magnitude and/or duration of effector cell responses. This functional diversification is often accompanied by changes in the expression of cell surface molecules. While the functional consequences directly attributable to the acquisition or loss of a particular membrane component are not always apparent, these changes may nonetheless serve as useful markers for discriminating cells that differ in function, lineage, or relative maturation.

[1]Department of Pediatrics, UCLA School of Medicine, Los Angeles, California 90024.

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Although a number of markers that define functionally distinct subpopulations of human T lymphocytes have now been identified, studies of the expression of isoforms of the CD45 common leukocyte antigen family have proven to be particularly rewarding. This review focuses on the ontogenetic relationship and functional heterogeneity of the reciprocal subsets of human T cells defined by the differential expression of two such isoforms, the CD45RA and CD45RO membrane antigens, and emphasizes the hypothesis that these T-cell subpopulations correspond to human "naive" and "memory" T cells. In addition, the significance of variations in the frequency of these subsets in selected clinical disorders is discussed.

BIOCHEMICAL CHARACTERISTICS OF CD45 ISOFORMS

The CD45 common leukocyte antigen family is comprised of a group of related membrane glycoproteins that are variably expressed by cells of lymphoid or myeloid origin. At least four isoforms, ranging in molecular mass from 220 to 180 kDa, have been identified (1). Although these isoforms share a common, large intracellular domain, differences in the composition of their extracellular domains, which are heavily glycosylated, are responsible for creating the unique antigenic determinants that distinguish these isoforms. It is currently unknown whether the differences in the extracellular portion of these isoforms have direct physiological consequences for the functions of T cells, and the nature of any ligands for the extracellular domains of the different isoforms is currently unknown.

Considerable progress has been made in defining the molecular basis for the generation of the different members of the CD45 antigenic family. These isoforms are produced by alternative mRNA splicing of three exons of a single gene found on chromosome 1 (2). Molecules expressing the CD45RA antigen have a relative molecular mass of 220 or 205 kDa and contain sequences near their amino terminus that are encoded by the A exon of this gene. In contrast, CD45RO molecules, which are 180 kDa in size (4), do not contain sequences encoded by exon A, B, or C. The differential use of these exons is responsible for variation in the primary amino acid sequences as well as secondary changes in the positions of carbohydrate linkages, both of which appear to be important in creating the antigenic variability of the different CD45 isoforms (1). Thus, monoclonal antibodies that recognize antigenic determinants specific for CD45RA molecules may react with either protease- or neuraminidase-sensitive epitopes (1, 3).

Although the precise physiological roles of the individual CD45 isoforms have not yet been identified, a number of recent studies have indicated that these molecules are involved in the regulation of transmembrane signals mediating lymphocyte activation. For example, mutagenized murine T cells which lack expression of any CD45 molecules are unable to proliferate in response to soluble antigens or anti-CD3 stimulation (5). Antibodies to CD45 molecules can inhibit a large number of immunological functions, including cell-mediated cytolysis, natural killer (NK) function, and B-cell proliferation and differentiation. Conversely, under certain conditions, anti-CD45 antibodies can upregulate IL-2 receptor expression by activated T cells or act as comitogens with anti-CD2 and anti-CD3 antibodies (6–8). An important step toward understanding the mechanism by which CD45 molecules may participate in lymphocyte activation was the demonstration that the intracellular domain of all members of this family has phosphotyrosine phosphatase activity (9). Recent studies have shown that CD45 isoforms may form complexes with different membrane molecules, including CD2 molecules on human T cells (10) and Thy-1 or CD3 molecules from murine T cells (11). Thus, one attractive hypothesis regarding the role of CD45 molecules in the regulation of lymphocyte activation proposes that CD45 molecules form complexes with membrane molecules involved in transmembrane signaling and enzymatically activate T-cell tyrosine protein kinases, such as $pp56^{lck}$ (12–14). It is currently unknown, however, whether differences in the extracellular portion of these isoforms influence the precise biochemical or physiological functions associated with the phosphotyrosine phosphatase activity of these molecules.

ONTOGENY AND TISSUE DISTRIBUTION OF THE T-CELL SUBSETS IDENTIFIED BY CD45 ISOFORM EXPRESSION

Analyses of the ontogeny of CD45RA and CD45RO antigen expression by T-lineage cells in the thymus have revealed several interesting findings. The CD45RO antigen is expressed by a majority of thymocytes, particularly those in cortical areas (15, 16). In contrast, the CD45RA antigen is

present on only a small subpopulation (10–15%) of relatively mature CD3+CD1−CD45RO− thymocytes that are concentrated in medullary areas (3, 15–17). These findings suggest that the intrathymic maturation of T-lineage cells may be accompanied by a switch from CD45RO to CD45RA isoform expression. The mechanisms responsible for inducing such a shift toward expression of the CD45RA antigen are presently unknown, although recent studies have suggested a role for interleukin-2 (IL-2) and/or IL-4 in this process (18). Interestingly, when CD45RA+ thymocytes are stimulated with anti-CD3 or anti-CD2 monoclonal antibodies, these cells acquire expression of the CD45RO antigen and cease to express the CD45RA antigen. This process is associated with fragmentation of cellular DNA and cell death by a process characteristic of apoptosis (19). Thus, it has been hypothesized that the CD45RA molecule may contribute to maintaining thymocyte viability and/or promoting the egress of CD45RA+ cells from the thymus (17). A corollary of this hypothesis is that thymocytes expressing the CD45RA molecule comprise the generative intrathymic lineage (20). The precise physiological roles of the CD45RA and/or CD45RO molecules during intrathymic T-cell differentiation remain to be determined, however.

Among circulating T cells, the vast majority (>90%) of CD4+ and CD8+ T cells present in cord blood of newborns coexpress CD45RA antigens (3, 21). During childhood, the relative frequency of circulating CD4+CD45RA+ cells declines (and the frequency of CD45RO+ cells increases) such that only 40–60% of circulating CD4+ T cells from adults are CD45RA+ (3, 22). This age-related decline in the frequency of CD45RA+ cells continues throughout adult life, although at a much less rapid rate than that seen during infancy and childhood (21).

The coexpression of the CD45RA molecule by CD4+ cells in secondary lymphoid tissues (lymph nodes, spleen) is considerably lower (10–15%) than seen for blood T cells (3, 15). Similarly, the CD4+ cells normally present in noninflamed tissues (including the lung, synovium, dermis, and lamina propria of the small intestine) are predominantly CD45RO+ cells (15, 23, 24). The basis for the preferential tissue location of CD45RO+ cells appears to be related to their increased expression of a variety of different membrane molecules capable of mediating cellular adhesion, as discussed below. Taken together, these observations suggest that the CD45RA antigen appears on T cells relatively late during their intrathymic differentiation and continues to be expressed by most of the relatively immature "virgin" or "naive" T cells present in neonates. With increasing age (and antigenic exposure), however, CD45RA−/RO+ cells become more prevalent in the circulation and comprise the majority of T cells in tissues.

FUNCTIONAL HETEROGENEITY OF CD45RA+ AND CD45RO+ T CELLS

Some of the most striking differences that have been described for CD45RA+ and CD45RO+ T cells have come from analyses of the functional capabilities of these subsets. Whereas CD4+CD45RA+ and CD4+CD45RA− cells have equivalent proliferative responses following their stimulation with allogeneic cells in the mixed lymphocyte reaction (MLR), CD4+CD45RA+ cells preferentially respond in the autologous MLR (22) and have greater proliferative responses to certain mitogens, such as phytohemagglutinin (PHA) (22, 25). CD45RA+ T cells also appear to be considerably more responsive to a number of cytokines, including IL-1, IL-2, IL-4, and IL-6, following their activation with anti-CD3 antibodies or the phorbol diester PMA (26, 27). In contrast, CD4+CD45RA− cells tend to respond more vigorously when stimulated with anti-CD3 antibodies (28, 29). This differential responsiveness is most apparent when low concentrations of anti-CD3 antibodies are used; at higher antibody concentrations, the responses of these subpopulations are virtually identical (30, 31). Similar results have been reported for the corresponding subsets of CD8+ cells (32). The response of these subsets to stimuli mediated by CD2 molecules is controversial. Whereas some workers have reported that CD45RA+ cells are more responsive to signals provided by mitogenic combinations of anti-CD2 antibodies (30), others have found the CD45RO+ subset to be superior in this regard (29, 33). Perhaps the most significant (and consistent) functional difference that has been found for these subsets comes from analyses of their ability to proliferate in response to soluble "memory" recall antigens; this capability appears to be unique to CD4+CD45RA− cells (4, 22, 25, 34).

Studies of the immunoregulatory functions performed by CD4+CD45RA+ and CD4+CD45RA− cells have shown that the ability to provide help for antibody production is performed uniquely by the

CD4+CD45RA− subset (4, 22, 34). The evidence reported to date indicates that this function (as determined in *in vitro* assays of human antibody production) is mediated by direct T–B-cell contact rather than via the elaboration of one or several lymphokines that effect B-cell differentiation (35). Although CD4+CD45RA− "memory" cells are uniquely able to provide help for B-cell differentiation, this subpopulation does not appear to perform all of the helper/inducer functions mediated by human CD4+ cells. For example, CD4+CD45RA+ and CD4+CD45RA− cells do not appear to differ in their ability to provide help for the generation of alloantigen-specific CD8+ cytotoxic T lymphocytes (CTL) in the MLR (36). This functional similarity appears to reflect the shared capacity of these subsets to produce IL-2, a factor which is critical for the generation of CTL, following their activation with allogeneic stimuli (36, 37). Thus, whereas the ability to provide help for antibody production is performed uniquely by CD4+CD45RA− cells, the myriad helper functions of human CD4+ cells are not homogeneous processes performed solely by the CD4+CD45RA− subpopulation.

The major immunoregulatory functions described for CD4+CD45RA+ cells involve suppression of immune responses. This may be accomplished by several mechanisms. First, CD4+CD45RA+ cells have been shown to be capable of inducing suppressor activity by CD8+ cells (22, 38). The mechanism underlying this process may directly involve the CD45RA molecule (38, 39). Whether this subset is uniquely capable of suppressor-inducer functions is controversial. Whereas some studies have reported that CD4+CD45RA+ cells are the only cells capable of mediating this function (22, 39), other have found that antigen-specific suppressor-inducer cells are present within the CD4+CD45RA− subset (40). In addition to inducing suppressor activity by CD8+ cells, CD4+CD45RA+ cells may also suppress at least some immune responses directly, without the participation of CD8+ T cells (41, 42). This function is particularly prominent in the CD4+CD45RA+ cells found in the immunologically naive human neonate. Numerous past studies have shown that cord blood CD4+ cells are deficient in their ability to provide help for antibody production. Although this functional deficit has been attributed to the presumed immaturity of CD4+ cells in the neonate, suppression by cord blood CD4+ cells has also been reported (43). Although >90% of the CD4+ cells in the neonate express the CD45RA+ phenotype, the small number of CD4+CD45RA− cells present in cord blood are capable of helper activity comparable to that of adult CD4+CD45RA− cells (44). However, this helper function is profoundly suppressed by the presence of even small numbers of radiation-sensitive cord blood CD4+CD45RA+ cells (44). The phenotypic properties of CD4+CD45RA+ cells in the neonate also differ from analogous cells in adults in that virtually all neonatal CD4+CD45RA+ cells coexpress the CD38 antigen (44). Thus, whereas cord blood CD4+CD45RA+ and CD4+CD45RA− cells share certain properties with the analogous subsets in adults, the dominant immunoregulatory function of cord blood CD4+ cells is suppression mediated by CD4+CD45RA+ cells. These data clearly indicate the need for caution in inferring the functional capabilities of CD4+ cells on the sole basis of CD45RA or CD45RO expression, without regard for other indicators of cell source and/or maturation.

The subsets of CD8+ cells defined by CD45 isoform expression also appear to be heterogeneous. Several studies have shown that both the CD8+CD45RA+ and the CD8+CD45RA− subsets contain precursor cells capable of differentiating into effector CTL when stimulated with alloantigens (39, 45, 46) or with immobilized anti-CD3 monoclonal antibodies (32). In contrast, CD8+ cells with cytotoxic effector functions (including those derived from CD45RA+ precursors) uniformly express the CD45RA− phenotype (45, 46). It is presently unclear whether CD8+ cells with suppressor functions are phenotypically homogeneous. Although the CD8+ suppressor cells generated in allogeneic or autologous MLR cultures uniformly express the CD8+CD45RA+ phenotype (39, 47), CD8+CD45RA+ and CD8+CD45RA− cells freshly prepared from peripheral blood are both capable of suppressing B-cell differentiation (45).

One possible basis for the differential functions of the subsets of T cells defined by CD45 isoform expression is that these subpopulations each produce a unique array of lymphokines, perhaps analogous to the patterns described for the Th1 and Th2 subsets of murine helper T cells (48). To date, there is little evidence suggesting that the subsets of human T cells identified by the expression of different CD45 isoforms correspond to these murine subsets. The preferential production of certain lymphokines (including IL-4 and IL-6) by human CD45RA− cells has been reported (46, 49), and this

subset appears to be the predominant, if not the sole, producer of interferon-γ (29, 50, 51). However, it is clear that cells within both subsets are capable of producing IL-2 when stimulated with allogeneic cells, high concentrations of anti-CD3 antibodies, or various other polyclonal activators (35, 37, 46, 50–52); indeed, several studies have shown that CD45RA+ cells are more proficient in producing this lymphokine (46), although the kinetics of IL-2 production may be slower for CD45RA+ cells (50). Of interest in this regard, the ability of CD8+ cells to undergo "helper cell-independent" differentiation into cytotoxic effector cells appears to be restricted to CD8+CD45RA+ cells as a result of the enhanced ability of this subset to produce and use IL-2 in an autocrine fashion (H. Bass, N. Yamashita, L. T. Clement, unpublished observations). Although the differential production of lymphokines remains an attractive hypothesis for explaining at least some of the functional heterogeneity of these T-cell subsets, there is no compelling evidence currently available indicating that this is the primary determinant of the functional differences between CD45RA+ and CD45RO+ T cells. This is particularly true with regard to the contrasting ability of these subsets to provide helper functions for B-cell differentiation and antibody secretion, a process that appears to require T–B-cell contact (35).

PHENOTYPIC HETEROGENEITY OF CD45RA+ AND CD45RO+ T CELLS

The subsets of T cells defined by CD45 isoform expression also differ in their expression of other membrane antigens. For example, cells comprising the CD45RA−/RO+ subset have increased expression of a variety of lymphocyte activation antigens, such as IL-2 receptors and class II major histocompatibility complex (MHC) antigens (46). Perhaps most notable in this regard is the heightened membrane expression by CD45RO+ T cells of a variety of adhesion molecules that mediate cell–cell interactions. These include the LFA-1 (CD18/CD11a), LFA-3 (CD58), CD2, and ICAM-1 (CD54) molecules, as well as the CD29 antigen, a member of the VLA family of integrins that is recognized by the 4B4 antibody (46, 51, 53). There is a growing body of evidence that the enhanced expression of these molecules influences the functional capabilities of CD45RO+ cells by promoting their binding to a variety of other cell types. Furthermore, it appears that the preferential homing and/or retention of CD45RO+ T cells in normal or inflamed tissues results from enhanced expression of these adhesion molecules by this subset (54). It should be noted, however, that the expression of some membrane proteins which function as adhesion molecules is more characteristic of CD45RA+ cells. Thus, the Leu-8/TQ1/LAM-1 antigen, an adhesion molecule that mediates lymphocyte binding to postcapillary high endothelial venules (55), is universally expressed by CD45RA+ cells yet is absent from 30–60% of CD45RA− cells (3). The expression of this molecule appears to regulate the recirculation pattern of CD45RA+ cells, which preferentially enter lymph nodes by crossing high endothelial venules (54).

LINEAL RELATIONSHIP OF THE SUBSETS EXPRESSING CD45R ISOFORMS

Two models of differentiation have been proposed to describe the lineal relationship of the T-cell subsets defined by CD45RA and CD45RO antigen expression. Because these phenotypically distinct cells perform different immunoregulatory functions, it has been hypothesized that these subsets represent mature, phenotypically and functionally stable progeny arising from separate differentiation pathways. According to this view, CD4+CD45RA+ cells comprise the suppressor-inducer subset, whereas cells with the CD4+CD45RA− phenotype are termed helper/inducer cells (22, 53). Alternatively, others have proposed that CD4+CD45RA− helper cells are "memory" cells that derive from "virgin" or "naive" CD4+CD45RA+ precursors via a common activation-dependent postthymic differentiation pathway (34, 56–59).

Two predictions emerge from the hypothesis that CD4+CD45RA+ and CD4+CD45RO+ cells represent "naive" and "memory" cells, respectively. First, if appropriately stimulated, CD4+CD45RA+ cells should be able to differentiate functionally and acquire helper functions. Second, this should result in their conversion to the CD45RA−/RO+ phenotype. It has been shown that CD4+CD45RA+ cells (from either adults or neonates) that are activated with PHA and subsequently cultured in IL-2 consistently acquire the ability to provide help for B-cell differentiation (44, 59). This functional differentiation is accompanied by a defined sequence of phenotypic changes. Less than 7% of freshly puri-

fied CD4+CD45RA+ cells coexpress either the CD29 or the CD45RO antigens. However, within 1–2 days of their activation, CD4+CD45RA+ cells rapidly acquire expression of both the CD29 and the CD45RO antigens, such that by day 3, >90% of the cells coexpress the CD29, CD45RO, and CD45RA antigens. Thereafter, with ongoing culture in IL-2, CD29 and CD45RO expression persists on >90% of the cells. In contrast, CD45RA antigen expression declines and is lost from all cells by day 10 (57–59). Comparable phenotypic changes have been described in association with the *in vitro* generation of "memory" alloreactive CD4+ cells (58), the generation of alloantigen-specific CD8+ cytotoxic effector cells (45), and the differentiation of antigen-specific helper cells for antibody production in the rat (60). Under these experimental conditions, this phenotypic conversion appears to be a permanent and unidirectional event, because (i) activated CD45RA+/RO− T cells (either in bulk culture or as individually cloned cells) uniformly convert to the CD45RA−/RO+ phenotype and (ii) activated CD45RA−/RO+ cells (either in bulk culture or as individually cloned cells) maintain their original CD45RA−/RO+ phenotype. Furthermore, within 24 hr after lymphocyte stimulation, the 5.4-kb mRNA species that encodes for the CD45RA isoform is no longer detectable (61). This also appears to be a unidirectional event; thus, the gradual loss of CD45RA antigen expression following lymphocyte activation appears to reflect a slow membrane turnover of this molecule. Finally, the pattern of lymphokine mRNA produced by activated CD45RA+ cells also changes; these cells acquire the ability to make lymphokines (IL-4 and interferon-τ) characteristically produced by the CD45RO+ subset (52).

Although the evidence cited above clearly demonstrates that CD45RO+ T cells can be derived from CD45RA+ precursors, several important issues merit attention. First, it should be noted that CD45RA antigen expression is not invariably lost from all activated and/or proliferating T-lineage cells. Transformed or malignant T-cell lines may remain CD45RA+ indefinitely (3, 62). Similarly, CD45RA antigen expression may persist on a minority of concanavalin A (Con A)-activated T cells under certain *in vitro* conditions (63), and a population of IL-4-responsive CD4+CD45RA+ cells present in cord blood can be grown in long-term cultures in the presence of IL-4 without permanent loss of CD45RA antigen expression (K. Kim, C. Uittenbogaart, and L. T. Clement, unpublished observations). The permanence and/or unidirectional nature of the loss of CD45RA isoform expression has also been questioned. It has been shown that the CD45RA antigen can be reexpressed by a small minority of cloned CD45RA− human T cells (64). Furthermore, expression of the 205-kDa (but not the 220-kDa) CD45RA isoform by human T cells can be induced or down-regulated in cyclic fashion under certain experimental conditions (65). Although these studies document that there are conditions under which at least some T cells can be activated without undergoing a total or permanent loss in their ability to express CD45RA antigens, these data do not obviate (or necessarily conflict with) those studies which clearly demonstrate that "naive" CD45RA+ cells activated by a variety of different stimuli can be induced to differentiate into "memory" CD45RO+ cells with helper functions under appropriate experimental conditions. Finally, it is important to note that data from *in vitro* studies may not reflect or recreate the complexities of *in vivo* T-cell differentiation. For example, studies in rats have shown that CD45RB+ T cells appear following the transfer of CD45RB− cells into athymic nude rats (66). Thus, it is possible that comparable *in vivo* changes in CD45RA isoform expression might occur in humans. The relevance of this provocative study of rodent CD45RB antigen expression to our understanding of the lineage of human T-cell subsets is clouded by the problem of interspecies dissimilarities in the expression of certain CD45 isoforms and the reagents available for their study. Data from a number of studies indicate that the pattern of CD45RB antigen expression by rodent T cells (as was analyzed in the aforementioned study) is not strictly analogous to that found for CD45RA antigens on human T cells (46). Furthermore, at least some monoclonal antibodies reactive with murine CD45RA antigens do not react with any murine T cells (S. Swain, personal communication). Accordingly, resolution of these important questions will require additional study.

ALTERATIONS OF T-CELL SUBSETS IN CLINICAL DISORDERS

Changes in the frequency of the subsets of T cells defined by CD45 isoform expression have been reported in a number of different clinical disorders. Perhaps most prominent have been the changes reported in a variety of inflammatory and/or autoim-

mune diseases. For example, there is a selective loss of circulating CD4+CD45RA+ cells in patients with multiple sclerosis (MS), particularly during disease exacerbations (67, 68). CD45RA+ cells are also selectively decreased in the lesions characteristic of MS (69). A decline in the frequency of circulating CD4+CD45RA+ cells has also been described in patients with systemic lupus erythematosus and renal disease (70). It is likely that this finding is of pathogenetic significance, because the magnitude of this reduction correlated with disease activity in these patients. Reductions in the relative frequency of circulating CD4+CD45RA+ cells have also been reported in patients with rheumatoid arthritis (71–73). These changes in circulating CD4+ cells are associated with a corresponding increase in the frequency of CD4+CD45RO+ cells in the exudate present in inflamed joints (72, 73). Although these changes could result from a variety of pathogenetic mechanisms, declines in the frequency of CD4+CD45RA+ cells with disease exacerbations might result from activation of "virgin" CD4+CD45RA+ cells and their accelerated *in vivo* differentiation to CD4+CD45RA "memory" cells, which then mediate the immunopathogenetic events of the disease processes. This hypothesis is consistent with the observation that the diminished frequency of CD4+CD45RA+ cells in MS patients occurs prior to clinical evidence of disease exacerbation (74). Alternatively, because most T cells in normal, noninflamed tissues express the CD45RO+ phenotype, the predominance of CD45RO+ cells in inflamed tissues could simply reflect increased migration and/or retention of this T-cell subset as a consequence of inflammation-induced changes in endothelial cell adhesion molecule expression (75). Neither of these alternatives precludes the possibility that a loss of cells with suppressor-inducer functions might also contribute to the disease process by allowing immune responses to proceed without feedback suppression.

Alterations of the frequency of CD45RA+ and CD45RO+ T cells have also been reported in a variety of immune deficiency disorders. Approximately 50% of patients with common variable immunodeficiency have diminished numbers of circulating CD45RA+ cells (76). Conversely, the memory CD4+CD45RO+ subset does not develop in patients with X-linked agammaglobulinemia (77) or the class II MHC deficiency syndrome (78), a disorder in which the lack of class II MHC antigen expression precludes normal antigen-induced T- and B-cell activation (79). Although there are a number of possible explanations for these findings, the failed development of T cells expressing the CD45RA−/RO+ phenotype in B cell-deficient individuals and in individuals whose circulating B cells lack class II antigens suggests that the principal accessory cells involved in the *in vivo* differentiation of naive CD45RA+ cells into CD45RO+ memory cells are HLA-DR+ B lymphocytes. Finally, subsets of CD4+ cells may be differentially involved in the pathogenetic processes leading to CD4-cell depletion in AIDS. Although the relative frequencies of circulating CD4+CD45RA+ and CD4+CD45RO+ cells decline at similar rates (80), recent studies have suggested that CD4+CD45RO+ cells may be preferentially infected and/or destroyed by the HIV-1 virus (81).

Interestingly, a few immunologically normal individuals have been identified in which virtually all their T cells express the CD45RA antigen *in vivo* as well as after *in vitro* stimulation (82). In the individuals with this genetic variant, however, subsets of CD4+ cells with normal functional segregation are defined by differential expression of the CD45RO antigen.

Finally, several recent studies have examined the properties of T cells arising after bone marrow transplantation (BMT) as a model system for assessing the factors that influence the generation of these phenotypically diverse subsets. The vast majority (>83%) of the CD4+ cells emerging after allogeneic BMT express the CD45RA− phenotype (83, 84). Most of these CD4+ cells also express activation antigens (HLA-DR, CD38). This subset imbalance persists throughout the first posttransplant year. Functionally, these CD4+CD45RA− cells are normal in their ability to provide help for antibody production. However, these memory cells do not mount a recall proliferative response to the tetanus antigen, even in instances where the marrow donor and recipient both had a vigorous response to this antigen prior to transplantation. Thus, the repopulation of CD4+ cells after BMT does not recapitulate the prenatal developmental pathway. Rather, it appears that the posttransplant predominance of CD4+CD45RA− cells may result from the activation and expansion of donor CD4+ T cells in the allogeneic environment of the recipient. These CD4+CD45RA− cells have normal helper capabilities but lack the appropriate immunological "memory" required for normal host defenses.

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