

Function of CD44(Pgp-1) homing receptor in human T cell precursors

Antonio de la Hera, Agustin Acevedo¹, Wendy Marston, and Francisco Sanchez-Madrid²

The Basel Institute for Immunology, Basel, Switzerland
Departments of ¹Pathology and ²Immunology, Hospital de la Princesa, Madrid, Spain

Key words: CD34⁺ stem cells, gp90 homing receptors, lymphocyte migration, T cell development, thymic stroma

Abstract

T cell precursors migrate from extrathymic hematopoietic tissues and differentiate after encountering the thymic microenvironment. We asked whether human T cell precursors express the CD44(Pgp-1/gp90HR) class of homing receptors that have been implicated in the traffic of hematopoietic cells, such as lymphocyte entry to peripheral lymphoid organs. Flow cytometry and immunoprecipitation studies demonstrate that CD7⁺34⁺, CD1⁻2⁻3⁻4⁻8⁻14⁻16⁻20⁻ cells in bone marrow and thymus, which have been shown to exhibit features of T cell precursors, bear CD44. Immunohistological studies show that clusters of thymocytes in the subcapsular and the inner cortex and most medullary thymocytes are clearly CD44⁺, whereas the expression of CD44 is selectively downregulated in CD3⁻ and CD3^{low} functionally incompetent cortical thymocytes. The expression of CD44 is not restricted to T cell precursors but also occurs in thymic stroma, which bear a different molecular species of CD44. CD44-specific antibodies exert stimulatory effects on T cell precursors, a process that is dependent on stromal cells. We postulate that CD44 might be an adhesion molecule for precursor homing to thymus and that it participates in cell-to-cell interactions within the thymic environment.

Introduction

T cell development occurs within a defined microenvironment, the thymus. Prethymic T cell progenitors develop in the fetal liver or postnatal bone marrow, circulate through the blood, and eventually home into the thymus. A few functionally competent thymocytes (~1%) emigrate to the periphery and recirculate through peripheral lymphoid organs (reviewed in refs 1–3).

Two families of gp90 homing receptors (hereafter referred to as gp90HRs) have been described (4–7). gp90HR^{Hermes} is indistinguishable from CD44(Pgp-1) antigen (8–16; this report). Although human gp90HR^{Hermes} shares MEL-14 antibody epitope with mouse gp90HR^{MEL-14} they are structurally distinct (7, 17–20). Both gp90HRs appear to mediate specific binding for endothelium and have therefore been implicated in T and B lymphocyte traffic (6,7). It is generally assumed that gp90HRs are acquired during B and T cell development in bone marrow and thymus respectively (6), and that CD44 in normal human thymus is associated with thymocyte functional maturation and location in the medulla (14).

In spite of this knowledge, very little is known about the role

of such receptors (and their ligands) in T cell precursor migration, commitment to T cell lineage, and intrathymic cell traffic. Here we entertain the hypothesis that acquisition of the gp90HR, as recognized by CD44 antibodies, precedes T cell differentiation in the thymus, and might play a role in early T cell development. We show that CD44 monoclonal antibodies (mAbs) bind to isolated pre- and intrathymic T cell precursors as well as thymic stromal cells, that CD44 is differentially expressed by mature and immature thymocytes, and that CD44 mAbs may exert stimulatory effects on T cell precursor and mature T cells.

Methods

Isolation of cells

Thymic lobes were obtained during corrective surgery and single cell suspensions were prepared. Bone marrow cells were from donor samples remaining after bone marrow transplantation. Pathology studies showed that all tissues used were normal.

Peripheral blood was from Kantonspital (Basel) blood bank donors.

Thymocytes and bone marrow cells expressing CD1, CD2, CD3, CD4, or CD8 T cell differentiation antigens, CD14⁺ myeloid cells, CD16⁺ natural killer cells, and CD20-bearing B cells were depleted by incubation with cytotoxic mAbs and rabbit complement, as described elsewhere (21). Viable cells not coated with mAbs were isolated, after incubation with M-450 magnetic beads armed with anti-mouse Ig antibodies, by Ficoll-Hypaque density centrifugation. The CD7⁺ cells were further separated from non-T lineage hematopoietic cells and stromal components using M-450 beads coupled with anti-CD7 mAbs and a sintered magnetic alloy. Cells were detached from the beads by incubation at 37°C. We followed the manufacturer's recommendation throughout the method. FACS analysis showed that 98% of the selected cells had the CD7⁺, CD1-2-3-4-8-14-16-20⁻ phenotype. Double-negative thymocytes were prepared by incubation with CD4 and CD8 mAbs and complement. The CD7-depleted population of thymocytes is a population highly enriched in thymic stromal cells, as determined with TE3A and TE4 mAbs specific for thymic epithelia. Thymic stroma was cultured in serum-free media supplemented with epidermal growth factor for 3 weeks to prevent the outgrowth of fibroblasts. After this time the cells (TE3/4+7⁻) were maintained in complete medium supplemented with 10% serum

Monoclonal antibodies

When possible, we will use the cluster of differentiation (CD) nomenclature proposed at the Oxford Workshop (8). CD1, Na1/34 (22); CD2, Leu-5 (Becton-Dickinson, Mountain View, CA); CD3, OKT3 (23; American Type Culture Collection, ATCC, Bethesda, MA); CD4, HP2/6 (24); CD7, 3A1 (ATCC), and RFT2 (25); CD8, B9.4 (26); CD14, CoulterCloneMo2 (Coulter Electronics, Hialeath, FL); CD16, Leu11b; CD19, CoulterCloneB4; CD20, CoulterCloneB1, and BC-1 (8); CD34, HPCA (Becton-Dickinson). OKT9 (transferin receptor, Ortho Pharmaceutical Co., Raritan, NJ), W6/32, TE3A, TE4, and TE7 (Serotec, Bicester, UK); Ki67 (27); and MEL-14 (5; ATCC).

The CD44 mAbs F-10-44-2 (9), 50B4 (10), and (Pgp-1) I42/5 (13) were generously provided by the authors (see Acknowledgements); A3D8 (15) was purchased from Serotec. The hybridoma clone HP2/9 (24) was generated by the fusion of P3X63 myeloma cells with spleen cells obtained from a mouse immunized with JM thymoma line (32). It produces an IgG₁ mAb. Because the nomenclature for the CD44 mAbs is quite fragmentary in the literature we shall briefly review it here. CD44 was defined by the 'cluster' of mAbs F-10-44-2, J-173, and 106-4D5 in the Third Workshop on Leukocyte Differentiation Antigens (8), but several other mAbs, such as those used above, can be formally considered as CD44-specific based on the results of Letarte (10). This author established that the gp85 molecular

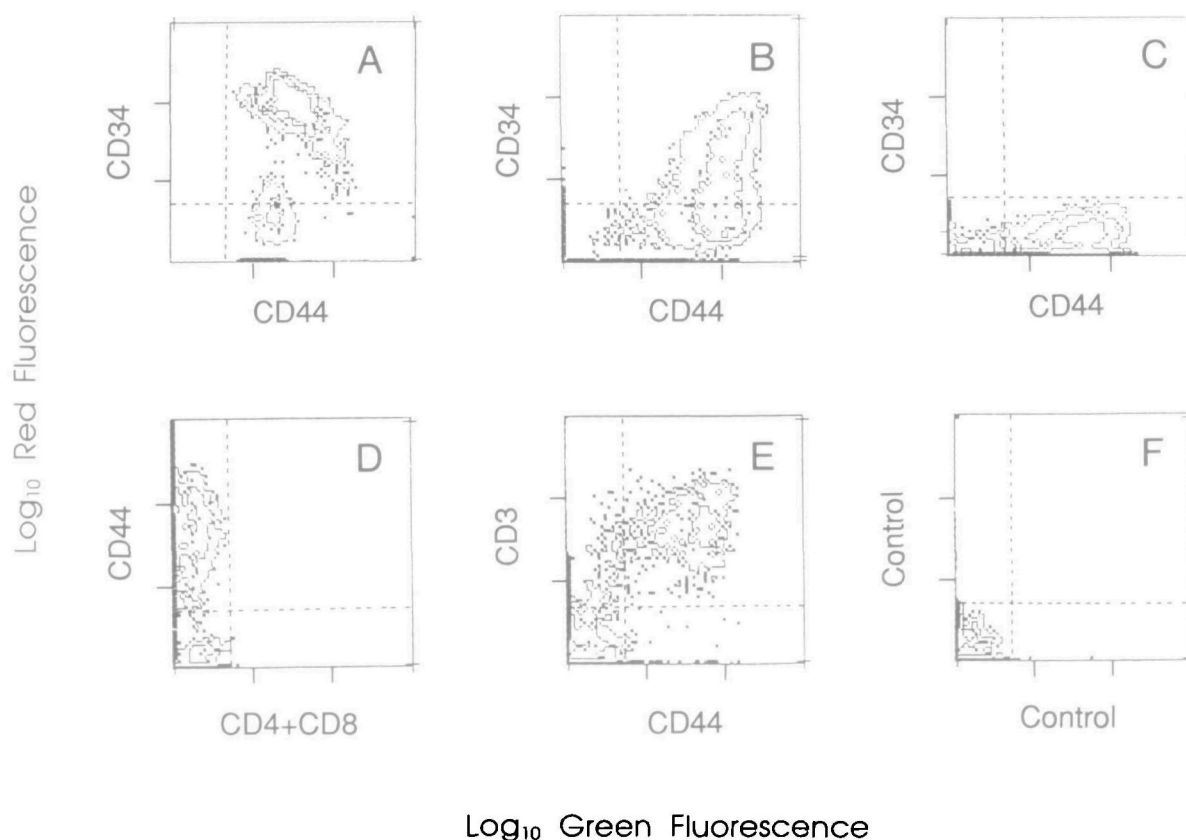


Fig. 1. CD44 is expressed in early T cell precursors and down-modulated during intrathymic development. Early T cell precursors from bone marrow (A), thymus (B), thymic epithelial cells (C), double-negative thymocytes (D), and total thymocytes (E) were isolated as described in Methods and stained with antibodies specific for the indicated antigens for two-color flow cytometry analyses. Antibodies were CD34 (HPCA, γ_1) and CD44 (50B4, γ_2) in the top panels, and CD44 (HP2/9, γ_1) and either CD4+CD8 (HP2/6 + B9.4, γ_2) or CD3 (OKT3, γ_2) in the bottom panels. Samples were analyzed in an EPICS flow cytometer equipped with a three-decade logarithmic amplifier

species exhibit at least three epitopes defined by the prototype mAbs F-10-44-2/A3D8/etc., 50B4/etc., and A1G3. Therefore, the brain-granulocyte T lymphocyte (9), p80 on mature thymocyte (14), and In(Lu) on erythrocyte (15) molecules all are recognized by CD44-specific mAbs. Moreover, anti-Pgp-1 mAbs have been independently shown to recognize the CD44 antigen (11,12). Also, in spite of their apparent relationship (9), CD44 and sialophorin are different molecular entities, the latter now termed CD43 (8,28)

Immunohistochemical staining of lymphoid organs

Acetone-fixed, 4- μ m-thick sections were sequentially incubated with mAb culture supernatants and peroxidase-conjugated rabbit anti-mouse Ig (Dakopatts, Copenhagen, Denmark); each incubation was followed by three washes. The reaction was

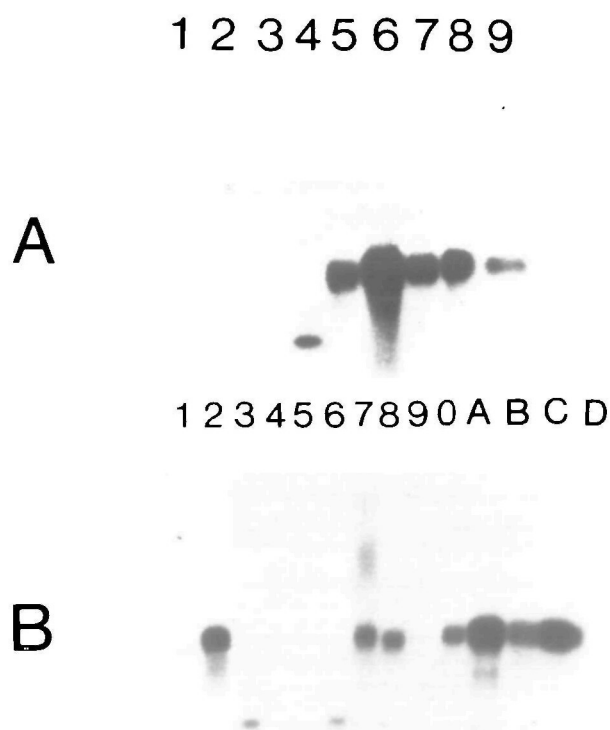


Fig. 2. Immunoprecipitation of CD44(Pgp-1/gp90HR) antigens from early thymocytes. Human early thymocytes, isolated from a pediatric thymus (2.5 years old) as described in Methods (21), were 125 I surface-labeled and the lysates immunoprecipitated with the following mAbs (A) Lane 1, CD2 + CD3 + CD4 + CD8 (anti-T cell mAbs); lane 2, CD20 (anti-B cell mAb); lane 3, CD14 + CD16 (anti-myeloid/NK cell mAbs); lane 4, CD7 (early thymocyte); lane 5, I42/5 (anti-Pgp-1); lane 6, F-10-44-2 (CD44); lane 7, 50B4 (CD44); lane 8, HP2/9, and lane 9, MEL-14 (gp90HR). (B) Lane 1, HP2/9 precipitate after preclearing/immunodepletion of the lysate with F-10-44-2; lane 2, HP2/9 undepleted; lane 3, CD7 undepleted; lane 4, MEL-14 precleared with HP2/9; lane 5, MEL-14 precleared with F-10-44-2; lane 6, CD7 precleared with HP2/9; lane 7, MEL-14 undepleted; lane 8, MEL-14 precleared with CD7; lane 9, MEL-14 precleared with anti-Pgp-1; lane 0, anti-Pgp-1 undepleted; lane A, F-10-44-2 undepleted; lane B, 50B4 undepleted; lane C, F-10-44-2 precleared with CD7; and lane D, F-10-44-2 precleared with HP2/9. Samples were subjected to electrophoresis and autoradiography for 16 and 12 days respectively

developed with Graham – Karnovsky medium containing 0.5 mg/ml of 3,3-diaminobenzimidine tetrahydrochloride (DAB) and hydrogen peroxide (29). Sections were mounted in buffered gelatin for microscopic examination.

Quantitative flow cytometry

The procedure for indirect immunofluorescence surface staining of cells has been described (21). For two-color analyses, IgG γ_1 and IgG γ_2 antibodies were used and labeled with either fluorescein isothiocyanate (FITC, green) or phycoerythrin-conjugated (PE, orange-red), second-layer, isotype-specific antibodies (Southern Biotechnology, Birmingham, AL). Quantitation of the staining of 10^4 (one-color) or 5×10^4 (two-color) viable cells was performed with either a Coulter EPICS-C cell-sorter or a FACscan instrument, as detailed elsewhere (21).

Immunoprecipitation and electrophoresis

Cells were radioiodinated with chloroglycoluril (Iodogen, Pierce, Rockford, IL), and lysed in phosphate-buffered saline, pH 7.4, containing 1% Triton X-100, 1% hemoglobin, and 1 mM phenylmethylsulfonylfluoride. For immunoprecipitation, 125 I-labeled cell lysates were incubated with 5 μ g of the indicated mAbs followed by rabbit anti-mouse or anti-rat Igs (Jackson Labs, ME) coupled to protein A – Sepharose (1 mg/ml). For immunodepletion, immunoprecipitation with the preclearing antibody was repeated twice before precipitation with the relevant antibody. Immunoprecipitates were processed and samples subjected to SDS-7 – 15% PAGE and autoradiography with enhancing screens, as described elsewhere (29).

Cell cultures

Cultures were maintained in RPMI 1640 medium supplemented with 2 mM glutamine, 10 mM HEPES, and 10% human serum (complement-depleted and pooled male AB donor), hereafter referred to as complete medium. For proliferative assays, 1.6×10^5 cells/well were cultured in 96-well microtiter plates in

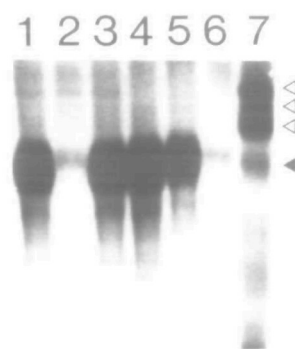


Fig. 3. Thymic epithelial cells express a different class of CD44 molecules. Peripheral blood mononuclear cells (1,3), lymph node cells (4), thymocytes (5), and thymic epithelial cells (2,6,7) were 125 I surface-labeled and the lysates precipitated with lane 1, 50B4; lane 2, 50B4 precleared with F-10-44-2; lanes 3, 4, 5, 7, F-10-44-2; and lane 6, F-10-44-2 precleared with 50B4. Samples were subjected to electrophoresis and autoradiography for 14 days. Filled arrowheads indicate the 'common' p85 band, open arrowheads show the multiple high-molecular-weight bands unique to thymic epithelial in the CD44 antibody precipitates from different sources

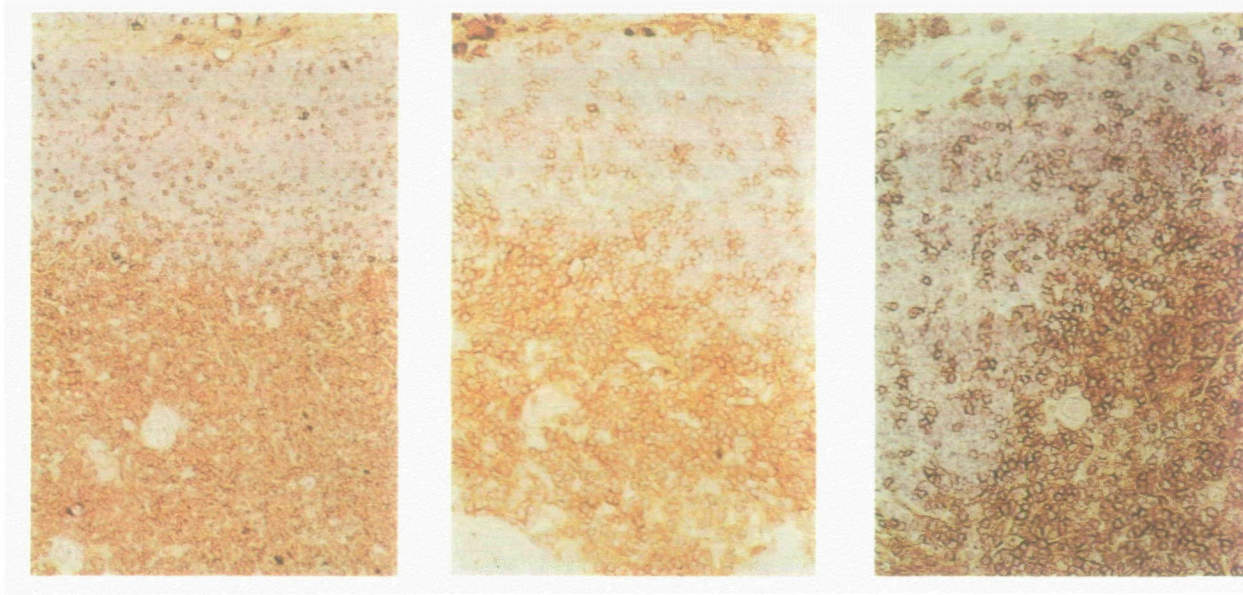


Fig. 4. Immunohistochemical staining shows binding of HP2/9 mAb to clusters of cortical thymocytes, most medullary thymocytes and thymic stromal components. Pediatric thymus sections were incubated with HP2/9 mAb and the staining was revealed by a DAB-peroxidase reaction as a brown precipitate, as described in Methods (29). Note that the thymic medulla is placed at the bottom while the outer cortex and the capsula are at the top in each panel. The results are indicated with three different thymus sections at increasing magnification from left to right.

complete medium supplemented with antibodies, 10 ng/ml phorbol myristate acetate (PMA), an ionophore (A23187, 1 μ M), or phytohemagglutinin (PHA, 1 μ g/ml), in the indicated combinations. The mAbs were directly bound to the plastic surface of the wells (1 μ g, 500 ng, 100 ng, and 10 ng/well), as described elsewhere (30). In some experiments early thymocytes were supplemented with autologous thymic stroma at a 1:80 ratio of thymic stroma to early thymocytes. Fresh stroma cells irradiated (2500 rad) were used in those complementation experiments. Limiting dilution analyses to determine the frequency of proliferating cells were performed after stimulation with PHA (1 μ g/ml) in the presence of 50 U/ml of interleukin-2 (IL2) and irradiated filler cells, as described by Lopez-Botet and Moretta (31). For IL2 production, 2×10^5 cells were cultured in the presence of PMA plus ionophore for 24 h. Supernatants were assayed for IL2 activity as described (30), using the IL2-dependent mouse CTLL2 cells. Human rIL2 (Hoffmann-LaRoche, Basel) was used as an internal control.

Results

CD44(Pgp-1/gp90HR) is expressed by early T cell precursors and thymic stroma

The expression of CD44 on T cell precursors was assessed by using immunofluorescence and flow cytometry. Normally, there are low numbers of T cell precursors in fetal liver, adult bone marrow, or the pediatric thymus (1,2). Early T cell precursors in humans have been characterized by both their unique phenotype (CD7⁺CD45⁺, CD1-2-3-4-8-) (33,34) and by their capacity to differentiate into either $\alpha\beta$ or $\gamma\delta$ TCR/CD3⁺ mature T cells in the presence of thymus stroma cells and IL2 *in vitro* (21,35). We isolated CD7⁺, CD1-2-3-4-8-14-16-20⁻ cells from bone

Table 1. CD44⁻ thymocytes are functionally unresponsive cells

	Frequency of proliferating cells	IL2 production (U/ml)
Peripheral blood mononuclear cells	1/147	21
Thymocytes		
Unseparated	1/1873	3
CD44 ^{high}	1/415	12
CD44 ^{intermediate}	1/4082	1
CD44 ⁻	<1/150	<0.1

The indicated cell populations were analyzed for the frequency of proliferating cells in limiting dilution assays in the presence of optimal amounts of PHA, IL2, and irradiated peripheral blood mononuclear cells (31). The IL2 production was measured in 24 h supernatants, after stimulation with PMA plus an ionophore, using an IL2-dependent mouse line (30). Thymocytes were separated into CD44(H2/9) high (the same level as peripheral blood lymphocytes), negative, and intermediate subsets using a fluorescence-activated cell sorter (31). Since the distinction between high and intermediate levels of CD44 expression is arbitrary (41), we cannot determine whether the few proliferating cells in the CD44^{intermediate} isolate were actually CD44^{low} cells or whether they represent contaminating CD44^{high} cells.

marrow and thymus and found that most of these cells express the CD44 molecule (Fig. 1A and B). Moreover, a sizeable fraction of them (1/3 in thymus and 2/3 in bone marrow) co-express CD34, a putative marker for hematopoietic stem cells (36; Fig. 1A and B).

We further explored the expression of the CD44(Pgp-1/gp90HR) antigens in early thymocytes using sequential immunoprecipitation studies with a panel of mAb that recognized four different distinct epitopes in the human CD44(Pgp-1/gp90HR) (5,10,13). As shown in Fig. 2(A), the material immunoprecipitated by all these mAb from lysates of

Table 2. CD44/gp90HR transduce co-mitogenic activation signals

	Proliferation (cpm × 10 ⁻³ /min) induced by								
	PMA – –	PMA ION –	PMA CD7 –	PMA CD44 –	PMA CD44 +AC	PMA Pgp-1 +AC	PMA – +AC	– CD44 +AC	PMA HLA-A,B +AC
Peripheral blood mononuclear cells	6	174	163	92	96	88	6	4	3
Early thymocytes	1	148	96	9	79	62	13	11	5

1.6 × 10⁵ cells were cultured in the presence of PMA, ionophore, solid-phase bound antibodies, and irradiated thymic accessory cells (AC) in the indicated combinations. F-10-44-2 (CD44), 142/5 (CD44/Pgp-1), 3A1 (CD7), and HLA-A,B (W6/32) antibodies were at 500 ng/well, PMA at 10 ng/ml, IONophore at 1 μM A23187, and the irradiated autologous thymic stroma at 2000 AC cells/well, which were found to be optimal in preliminary experiments (30; unpublished results). Proliferation was measured after 3 days by adding 1 μCi [methyl-³H]thymidine (Radiochemical Centre, Amersham) per well for the last 12 h of culture. Results represent the mean [³H]thymidine uptake in triplicate cultures of an experiment representative of three.

surface-labeled human early thymocytes migrated on SDS-PAGE with a near-identical mobility at ~85 kd. Also, CD7 mAb but not mAbs specific for T-, B-, and myeloid-differentiation antigens immunoprecipitated labeled material at ~40 kd, consistent with the early thymocyte phenotype of the population used (21,33,34). We confirmed that HP2/9 (32) is a CD44 mAb as shown by reciprocal pre-clearing in immunodepletion experiments. Moreover, HP2/9 mAb specifically immunodepletes the precipitates of the prototype gp90HR mAb, MEL-14, as do CD44 and Pgp-1 mAbs (Fig. 2B). These results are consistent with the recently established co-identity of CD44, gp90HR^{Hermes}, and Pgp-1 (11,12,16). We conclude that human early thymocytes express CD44(Pgp-1/gp90HR) homing receptors as assessed by both quantitative flow cytometry and biochemical methods.

Interestingly, we found that thymic stromal cells express CD44. Freshly isolated thymic stromal cells were CD44⁺ but lacked the CD34 molecule (Fig. 1C). These cells remained CD44⁺ in culture, which allowed us to characterize biochemically the CD44 molecule they expressed. As shown in Fig. 3, the nature of the CD44 molecules expressed on thymocytes, tonsil lymphocytes, and peripheral blood mononuclear cells was roughly identical, but strikingly different from CD44 expressed on thymic stroma. In the latter cells, additional high-molecular-weight bands besides the 90 kd species found on T cell precursors are apparent. All these bands were immunodepleted by two CD44 mAb specific for different epitopes (10), indicating that either the additional bands carry the two CD44 determinants, or they are non-covalently associated to the 80–90 kd band.

CD44 expression is downregulated during intrathymic development in functionally incompetent cells

The level of CD44 surface expression is regulated during thymic ontogeny. For instance, only a portion of the CD4⁺8[–] 'double-negative' thymocytes express CD44 (Fig. 1D). Based on expression of CD3 and CD44, four major subsets of thymocytes were evident: CD3[–]CD44[–], CD3^{dim}CD44[–], CD3^{low}CD44^{low}, and CD3^{high}CD44^{high} (Fig. 1E). Minor subsets of CD3[–]CD44⁺ and CD3^{low}CD44^{high} were also detected, which comprise some double-negative thymocytes and the T cell precursors (Fig. 1A and B; see above). We also studied reactivity of CD44 mAb on tissue sections of thymus using immunohistochemical staining. Most cortical thymocytes are unstained (Fig. 4, left panel). Most medullary thymocytes as well as some scattered thymocytes in the subcapsular and inner cortex are positive in thymus sections

The CD44^{high} cortical thymocytes are aggregated in several independent clusters of 2–9 cells (Fig. 4, right panel).

The fact that CD44[–] thymocytes are localized in the cortex and lack CD3 suggested that they could be immature thymocytes. By using limiting dilution analyses of the frequency of proliferating cells, and measuring the production of IL2 in bulk cultures, we found that CD44[–] thymocytes were indeed inert cells while the CD44^{high} population contained functional thymocytes. The CD44^{intermediate} population contained some fewer functional cells (Table 1).

CD44 could participate in T cell precursor activation

We have studied whether CD44 might be an immunoadhesin involved in the activation of T lymphocytes. CD44 mAbs by themselves were not mitogenic for peripheral blood lymphocytes. Activation of T cells with mAbs directed to accessory molecules such as CD2, CD4, and CD8 usually require co-stimulatory signals (30,37). PMA along with mAbs against different surface antigens can elicit T cell proliferation (30). We found that the CD44(Pgp-1)-specific mAbs F-10-44-2 and 142/5 together with PMA were able to stimulate peripheral blood lymphocytes to proliferate (Table 2). The effect of CD44 mAbs was specific and dependent on the amount of antibody added. We were also interested in studying the effect of CD44-specific mAb on human early thymocytes. The results depicted in Table 2 show that early thymocytes proliferate in response to PMA + A23187 stimulation. The CD44-specific mAbs can substitute for the effect of ionophore in the presence but not in the absence of thymic stroma 'accessory cell' (AC). The effect was observed with CD7-specific mAb which did not require accessory cells to promote proliferation (30), whereas anti-HLA A,B,C mAb did not exhibit such activity. At present it is not clear whether the effect of CD44-specific mAbs is mediated directly at the level of the T cell precursors, via thymic stromal cells, or both.

Discussion

We have demonstrated that CD44 is expressed on CD7⁺, CD1[–]2[–]3[–]4[–]8[–]14[–]16[–]20[–] cells in the thymus and bone marrow and that a sizeable portion of these cells also bears CD34. CD34 is an antigen present on 1–2% bone marrow cells that include the pluripotent hematopoietic stem cells (36), hence these results suggest that CD44 may be already expressed by the pluripotent stem cells or prethymic T cell precursor stage. We

also show that clusters of thymocytes in the cortex and most medullary thymocytes are also CD44⁺ and that the expression of CD44 is significantly decreased or switched-off on the CD3^{low-to-negative} cortical thymocytes. Since early thymocytes and peripheral T cells bear CD44, we conclude that CD44 expression is down-regulated during intrathymic development in functionally inert thymocytes. This interpretation would imply that CD44 is not acquired late in the T cell ontogeny by medullary and functionally mature thymocytes (14,33). CD44-related homing receptors do not therefore show a different distribution in human and other species: (i) mouse pluripotent stem cells and pro-thymocytes express Pgp-1 (13); (ii) fetal thymocytes from mouse express MEL-14 and both mouse and sheep express Pgp-1 (11, 38,39); and (iii) subsets of immature and mature adult mouse thymocytes were found to be Pgp-1⁺ or MEL-14⁺ (11,40,41).

We have shown that thymic stromal cells express several different molecular forms of CD44, all of which can be immunodepleted by CD44 mAbs defining two distinct epitopes. Our results agree with the findings that CD44 may be either a multigene family or present in multiple exons being expressed in two distinct patterns in epithelial and hematopoietic cells (33; M. Letarte, personal communication). The above results uncover a homing function for CD44(Pgp-1) receptors. As endothelial cells also bear CD44, we can postulate that CD44 is expressed not only on the migrating cell (6,7) but also at the surface of cells that may direct homing processes (i.e. endothelia or thymic stroma).

We also found here that CD44-specific antibodies exert co-stimulatory effects on the proliferation of T cell precursors, a phenomenon that is dependent on stromal cells. Since both T cell precursors and thymic stroma express CD44, we could not delineate the mechanisms underlying the effect of the anti-CD44 mAbs. The mechanism by which accessory cells participate in the CD44 co-mitogenic effects on T cells needs to be studied, especially as thymic stroma is normally required for T cell differentiation (1–3,35). CD7⁺, CD1-2-3-4-8-14-16-20-early thymocytes can be expanded using polyclonal activators in the absence of thymic stroma without differentiation. These cells retain CD44 expression and CD44-specific antibodies interfere in their binding with thymic stroma (unpublished results). Cell-to-cell interaction through different adhesion molecules may be a source of information, along with growth factors and other environmental signals, that a cell precursor receives and translates into the appropriate growth movement, and differentiation response (42). In this view, the possibility that CD44 could be an adhesion (homing) receptor does not preclude the possibility that CD44 molecules could also be implicated in T cell differentiation. Both cell-adhesion and movement functions of CD44 are well documented in other cell types (7,43). Also, as shown here for CD44, other surface receptors are involved in cell adhesion and cell activation, such as CD2 and its ligand LFA3, or LFA-1 (30,37). In the B cell lineage, the LFA-1 integrin may be involved in the differentiation process of PRO-B cells promoted by bone marrow stroma cells (44). Along these lines, we postulate that CD44 could play a role as an adhesion molecule for T cell precursor homing to the thymus and might perhaps participate in some precursor – stromal cell interactions occurring during early T cell activation for proliferation and differentiation.

Acknowledgements

We thank Beat Imhof, Charles Mackay, P. Matzinger, and R. Palacios for critical reading of the manuscript, the Thoracic Surgery Unit, Kantonsspital, Basel, and the Paediatric Cardiosurgery Unit, CERyC, Madrid, for the thymus samples; Dr Alvarez-Mon for the bone marrow samples, Drs R. Dalchau, G. Jannossy, M. Letarte, C. Mawas, T. Springer, I. Trowbridge, and J. Vives for the antibodies; Dr F. Sinigaglia for the rIL2; B. Pfeiffer and H. Spalinger for the artwork; and D. Thorpe for technical assistance. The Basel Institute was founded and is fully supported by Hoffmann-LaRoche & Co. Ltd, Basel, Switzerland. This work was partially supported by CICYT and FISS. A.H. is on leave of absence from the Centro de Investigaciones Biológicas, CSIC, Madrid.

Abbreviations

CD	cluster of antibodies directed to differentiation antigens
DBA	diaminobenzimidine tetrahydrochloride
FITC	fluorescein isothiocyanate
gp90HR	a class of 90 kd homing receptors
IL2	interleukin-2
PE	phycoerythrin
PHA	phytohemagglutinin
PMA	phorbol myristate acetate
TCR	T cell receptor

References

- 1 Toribio, M. L., Alonso, J. M., Barcena, A., Gutierrez, J. C., de la Hera, A., Marcos, M. A. R., Marquez, C., and Martinez-A, C. 1988. Human T-cell precursors: involvement of the interleukin 2 pathway in the generation of mature T cells. *Immunol. Rev.* 104:55
- 2 Palacios, R. and Pelkonen, J. 1988. Prethymic and intrathymic T-cell progenitors. *Immunol. Rev.* 104:5
- 3 MacDonald, H. R., Howe, R. C., Pedrazini, T., Lees, R. K., Budd, R. C., Schneider, R., Liao, N. S., Zinkernagel, R. M., Louis, J. A., Raulet, D. H., Hengartner, H., and Miescher, G. 1988. T cell lineages, repertoire selection and tolerance induction. *Immunol. Rev.* 104:157
- 4 Jalkanen, S. T., Bargatze, R. F., Herron, L. R., and Butcher, E. C. 1986. A lymphoid cell surface glycoprotein involved in endothelial cell recognition and lymphocyte homing in man. *Eur. J. Immunol.* 16:1195
- 5 Gallatin, M., Weissman, I. L., and Butcher, E. C. 1983. A cell-surface molecule involved in organ-specific homing of lymphocytes. *Nature* 304:30
- 6 Gallatin, M., St. John, T. P., Siegelman, M., Reichert, R., Butcher, E. C., and Weissman, I. L. 1986. Lymphocyte homing receptors. *Cell* 44:673
- 7 Butcher, E. C. 1986. The regulation of lymphocyte traffic. *Curr. Top. Microbiol. Immunol.* 128:85
- 8 McMichael, A. J. 1987. In *Leukocyte Typing III*. Oxford University Press, Oxford
- 9 Dalchau, R., Kirley, J., and Fabre, J. W. 1980. Monoclonal antibody to a human brain-granulocyte-T lymphocyte antigen probably homologous to the W3/13 antigen of the rat. *Eur. J. Immunol.* 10:745
- 10 Letarte, M. 1986. Human p85 glycoprotein bears three distinct epitopes defined by several monoclonal antibodies. *Mol. Immunol.* 23:639
- 11 Mackay, C. R., Maddox, J. F., Wijffels, G. L., Mackay, I. R., and Walker, I. D. 1988. Characterization of a 95,000 molecule on sheep leucocytes homologous to murine Pgp-1 and human CD44. *Immunology* 65:93
- 12 Omary, M. B., Trowbridge, I. S., Letarte, M., Kagnoff, M. F., and Isake, C. M. 1988. Structural heterogeneity of human Pgp-1 and its relationship with p85. *Immunogenetics* 27:460
- 13 Trowbridge, I. S., Lesley, J., Schulte, R., Hyman, R., and Trotter, J. 1983. Biochemical characterization and cellular distribution of a

- polymorphic, murine cell-surface glycoprotein expressed on lymphoid tissues. *Immunogenetics* 15:299.
- 14 Haynes, B. F., Harden, E. A., Telen, M. J., Hemler, M. E., Strominger, J. E., Palko, T. J., Searce, R. M., and Eisenbarth, G. S. 1983 Differentiation of human thymocytes. I. Acquisition of a novel human cell surface protein (p80) during normal intrathymic T cell maturation. *J Immunol* 131:1195
 - 15 Telen, M. J., Eisenbarth, G. S., and Haynes, B. F. 1983. Human erythrocyte antigens. Regulation of expression of a novel erythrocyte surface antigen by the inhibitor Lutheran *In(Lu)* gene. *J Clin. Invest* 71:1878.
 - 16 Picker, L. J., de los Toyos, J., Telen, M., Haynes, B. F., and Butcher, E. C. 1989 Monoclonal antibodies against the CD44 [In(Lu)-related p80], and Pgp-1 antigens recognize the hermes class of lymphocyte homing receptors. *J Immunol* 142:2046
 - 17 Stamenkovic, I., Amiot, M., Pesando, J. M., and Seed, B. 1989 A lymphocyte molecule implicated in lymph node homing is a member of cartilage link protein family. *Cell* 55:1057
 - 18 Goldstein, L., Zhou, D. F. H., Picker, L. J., Minty, C. N., Bargatze, R. F., Ding, J. F., and Butcher, E. C. 1989 A human lymphocyte homing receptor, the Hermes antigen, is related to cartilage proteoglycan and link proteins. *Cell* 55:1063.
 - 19 Siegelman, M. H., van de Rijn, M., and Weissman, I. L. 1989 Mouse lymph node homing receptor cDNA clone encodes a glycoprotein revealing tandem interaction domains. *Science* 243:1165.
 - 20 Lasky, L. A., Singer, M. S., Yednock, T. A., Dowbenko, D., Fennie, C., Rodriguez, H., Nguyen, T., Stachel, S., and Rosen, S. D. 1989 Cloning of a lymphocyte homing receptor reveals a lectin domain. *Cell* 55:1045
 - 21 Toribio, M. L., de la Hera, A., Borst, J., Marcos, M. A. R., Marquez, C., Alonso, J. M., Barcena, A., and Martinez-A., C. 1988. Involvement of the interleukin 2 pathway in the rearrangement and expression of both $\alpha\beta$ and $\gamma\delta$ T cell receptor genes in human T cell precursors. *J. Exp Med.* 168:2231
 - 22 McMichael, A. J., Pilch, J. R., Galfre, G., Masson, D. Y., Fabre, J. W., and Milstein, C. 1979. A human thymocyte antigen defined by a hybrid myeloma monoclonal antibody. *Eur J Immunol* 9:205
 - 23 Reinherz, E. L. and Schlossman, S. F. 1980. Discrete stages of human intrathymic differentiation analysis of normal thymocytes and leukemia lymphoblasts of T cell lineage. *Cell* 19:821
 - 24 Sanchez-Madrid, F., O. de Landazuri, M., Morago, G., Cebrian, M., Acevedo, A., and Bernabeu, C. 1986 VLA-3: a novel polypeptide associated within the VLA molecular complex cell distribution and molecular characterization. *Eur J Immunol.* 16:1343.
 - 25 Campana, D. and Janossy, G. 1986 Leukemia diagnosis and testing of complement fixing antibodies for bone marrow purging in ALL. *Blood* 68:1264
 - 26 Malissen, B., Rebai, N., Liabeuf, A., and Mawas, C. 1982 Human cytotoxic T cell structures associated with the expression of cytotoxicity. *Eur J Immunol* 15:88.
 - 27 Gerdes, J., Lemke, H., Baisch, H., Wacker, H. M., Schwab, V., and Stein, H. 1984 Cell cycle analysis of a cell proliferation-associated human nuclear antigen defined by the monoclonal antibody Ki-67. *J. Immunol.* 133:1710.
 - 28 Borche, L., Lozano, F., Vilella, R. and Vives, J. 1987. CD43 monoclonal antibodies recognize the large sialoglycoprotein of human leukocytes. *Eur. J Immunol.* 17:1523.
 - 29 Pulido, R., Cebrian, M., Acevedo, A., O. de Landazuri, M., and Sanchez-Madrid, F. 1988. Comparative biochemical and tissue distribution study of four distinct CD45 antigen specificities. *J Immunol* 140:3851
 - 30 Carrera, A. C., Rincon, M., Sanchez-Madrid, F., Lopez-Botet, M., and O. de Landazuri, M. 1988. Triggering of co-mitogenic signals in T cell proliferation by anti-LFA-1 (CD18, CD11a), LFA-3, and CD7 monoclonal antibodies. *J Immunol.* 141:1919
 - 31 Lopez-Botet, M. and Moretta, A. 1985 Functional analysis of human thymocytes. *J. Immunol* 134:2299
 - 32 de la Hera, A., Alvarez-Mon, M., Sanchez-Madrid, F., Martinez-A., C., and Durantez, A. 1988 Co-expression of Mac-1 and p150,95 on CD5+ B cells. *Eur J Immunol* 18:1131
 - 33 Lobach, D. F. and Haynes, B. F. 1987. Ontogeny of the human thymus during fetal development. *J. Clin Immunol* 7:81
 - 34 Campana, D., Janossy, G., Coustan-Smith, E., Amiot, P. L., Tian, W.-T., Ip, S., and Wong, L. 1989 Expression of the T cell receptor associated proteins during T cell ontogeny in man. *J Immunol.* 142:57
 - 35 de la Hera, A., Marston, W., Aranda, C., Toribio, M. L., and Martinez-A., C. 1989 Thymic stroma is required for the development of human T cell lineages *in vitro*. *J. Immunol.* 1 in press
 - 36 Berenson, R. J., Andrews, R. G., Besinger, W. I., Kalamasz, D., Knitter, G., Buckner, C. D., and Bernstein, I. D. 1988 Antigen CD34+ marrow cells engraft lethally irradiated baboons. *J Clin Invest* 81:951
 - 37 Meuer, S. C., Hussey, R. E., Fabbri, M., Fox, D. A., Acuto, O., Fitzgerald, K. A., Hodgdon, R. C., Protentis, J. P., Schlossman, S. F., and Reinherz, E. L. 1984 An alternative pathway of T-cell activation a functional role for the 50KD T11 sheep erythrocyte receptor protein. *Cell* 36:897.
 - 38 Lynch, F. and Ceredig, R. 1988 Ly-24(Pgp-1) expression by thymocytes and peripheral T cells. *Immunol Today* 9:7
 - 39 Reichert, R. A., Weissman, I. L., and Butcher, E. C. 1986 Phenotypic analysis of thymocytes that express homing receptors for peripheral lymph nodes. *J. Immunol.* 138:343.
 - 40 Budd, R. H., Cerottini, J. C., Horvath, C., Bron, C., Pedrazzini, T., Howe, R. C., and MacDonald, H. R. 1987 Distinction of virgin and memory T lymphocytes. *J Immunol* 138:3120.
 - 41 Shortman, K., Wilson, A., van Ewijk, W., and Scollay, R. 1987. Phenotype and localization of thymocytes expressing the homing receptor-associated antigen MEL-14 arguments for the view that most mature thymocytes are located in the medulla. *J. Immunol.* 138:342
 - 42 Barnstable, C. J. 1986 Clues about glues in development. *Nature* 321:731.
 - 43 Jacobson, K., O'Dell, D., Holfield, B., Murphy, T. L., and August, J. T. 1984. Redistribution of a major cell surface glycoprotein during cell movement. *J Cell Biol* 99:1613
 - 44 Kinashi, T., Inaba, K., Tsubata, T., Tashiro, K., Palacios, R., and Honjo, T. 1988. Differentiation of an interleukin 3-dependent precursor B-cell clone into immunoglobulin-producing cells *in vitro*. *Proc Natl Acad Sci USA* 85:4473