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Lymphocyte and Lymphoma Receptors Utilized in Differentiation, in Homing, and in Lymphomagenesis *

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A. Normal Thymic Maturation

The thymus is believed to be the major, if not the sole site of differentiation of T lymphocytes [1, 2]. During fetal development the thymus receives a bolus of precursors of T cells from the hematopoietic organs, probably the fetal liver and/or the yolk sac [3, 4]; these populations subsequently undergo self-renewal as well as maturation [5]. In adult life the thymus receives cells from the bone marrow at a very low level, but in times of stress, or after irradiation, there is a massive renewal of cells in the thymus from bone marrow precursors [2].

The thymus is divisible into a cortex and a medulla. The cortex contains about 85% of the total lymphocyte population, whereas the medulla harbors the remaining 15%. The outer cortical region has a layer of self-renewing blast cells that also gives rise to the majority if not all of the cells in the cortex, as well as to a population that resides in the medulla [1, 6]. The thymus turns over its lymphoid population (at least in the cortex and partially in the medulla) every 3–5 days [1, 2]. Yet the thymus exports only about 1% of the cells in the thymus per day at 4 weeks of life, and 0.1% of the total thymic content at 6 months of age [5].

The "conventional wisdom" about thymus maturation is that the cortex contains functionally immature lymphocytes and that medullary cells are the functionally mature population [7]. Treatment of an animal with cortisone results in disappearance of the lymphoid cortex, while the medulla appears to remain intact. The cell types that are present in the medulla after cortisone share surface phenotypic markers (PNA^{lo}, $H\text{-}2K^{hi}$, TL^{-}) with medullary cells in the intact thymus, and it has been assumed that cortisone-resistant thymocytes = medullary thymocytes = immunocompetent T cells. Here we demonstrate that this notion is wrong, and that what really happens is more complex.

Peripheral, recirculating, immunocompetent T and B lymphocytes bear cell-surface *homing receptors* which specify their adherence to specialized cells lining high-walled endothelial venules (HEV) in peripheral lymphoid organs [8]. At least two *independent* homing receptors have been characterized, one for Peyer's patch HEV and one for lymph node HEV [8]; the latter contains an epitope detected by the monoclonal antibody MEL-14 [9]. In contrast to most peripheral lymphocytes, only 2%–5% of thymocytes are capable of homing to the periphery or binding to the HEV of these peripheral lymphoid organs. The fluorescence-activated cell sorter profile of MEL-14-stained thymocytes showed that about 3% of the cells in the thymus stained brightly and at levels equivalent to most peripheral T cells, while about 85% of the cells stained at 10% of that amount per cell and the remaining 5%–15% had no detectable stain. Where are these homing-competent thymocytes located? According to conventional wisdom, they should be in the medulla.

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Immunohistochemical analysis of thymic frozen sections reveal, contrary to conventional wisdom, that the MEL-14^{hi} cells in the thymus are in the cortex rather than in the medulla. Do these cells give rise to the thymus cell migrants? Direct intrathymic infusion with fluorescein isothiocyanate labels thymic cells randomly, and allows detection of thymic emigrants. The cells that had homed into the lymph nodes or to the spleen were at least 80%–85% MEL-14^{hi}, and they came from a population of cells (that is, the thymus) in which only 1%–3% of the cells stained at such levels. Since it occurred within 3 h after intrathymic infusion, we concluded that the cells in the thymus which give rise to the major population of emigrants are the MEL-14^{hi} *cortical* cells. The MEL-14^{hi} cells within the thymus are mainly PNA^{lo} and H-2K^{hi}, similar to peripheral T cells (and medullary cells) [10]. Thus, MEL-14^{hi} thymocytes are cortical in location yet express the mature T cell phenotype. To test the *functional* capability of these MEL-14^{hi} thymic cells we did limiting dilution analysis of thymocytes to test their ability to recognize an MHC class I alloantigen by giving rise to clones of cytolytic T cells. The overall frequency of thymic cells which can give rise to allospecific cytolytic clones is about 1 : 10 000 cells. In the MEL-14-highest 2% of cells in the thymus it is 1 : 600, in the MEL-14-lowest 50% of cells, less than 1 : 100 000 [11]. Thus, most of the thymic allospecific cytolytic T cell precursors are MEL-14^{hi} cortical cells.

It appears that the cells in the thymus which are capable of responding to antigen and are capable of giving rise to thymus cell migrants are in the cortex rather than in the medulla. How can we resolve the paradox that 2–5 days after cortisone treatment the thymus contains only a population of cells restricted to the medulla which are immunologically competent, which when put into the bloodstream will home perfectly well, and which includes precursors of killer cells and of helper cells? Following cortisone treatment the *percentage* of cells that are MEL-14^{hi} increases dramatically, but the absolute number of MEL-14^{hi} cells only changes slightly [10]. We proposed that the MEL-14^{hi} cells in the cortex are cortisone-resistant and that most of the other cells in the thymic cortex are cortisone-sensitive. In fact, the thymus of hydrocortisone-treated mice undergoes massive pyknosis for 1–2 days, but among the cells in the thymus which survive are the MEL-14^{hi} subset. By 2 days the surviving MEL-14 cells reside in the medulla [10]. While it seems likely that MEL-14^{hi} cells in the cortex account for a significant proportion of the cortisone-resistant cells which end up in the medulla, we cannot rule out the possibility that there are some cortisone-resistant cells in the medulla which respond to hydrocortisone by becoming MEL-14^{hi} and immunologically competent.

The major set of problems that we have to solve next about thymus cell maturation has to do with *determination* of cells. At what point in their life history do thymic lymphocytes (or their precursors) become *determined* to express a particular surface phenotype (e.g., T4 or T8 in man, or L3T4 or Lyt-2 in the mouse)? At what stage(s) do they rearrange and express the T cell antigen receptor α- and β-chain genes? If these events occur before T cell precursors enter the thymus, the thymus will be mainly a selective microenvironment, whereas if they occur after arriving in the thymus the thymus could also be involved in generating receptor diversity amongst a population of noncommitted cells. At what point do they gain MHC restriction? Self/non-self discrimination? When do they commit to function (help, kill, suppress)? For all these questions we need a way of isolating clones of pre-T cells to follow their progeny in terms of each of these various commitments. We think we have found a way to clone them in vivo. To do this we combine small numbers of bone marrow cells from mice that are genetically Thy-1.2 with a large number of bone marrow cells from mice that are genetically Thy-1.1, and inject them into lethally irradiated mice. The Thy-1.2 cells are present in limiting numbers to allow rare thymus-entering cells to give rise to clonal outgrowths within these thymuses. We identify such clonal outgrowths by immunohistochemical localization of Thy-1.2 foci in host thymuses 3 weeks to 6 months after injection. In

80%–90% of focal (presumably clonal) repopulations, the focus spans both cortex and medulla. In 6/85 cases we found a medulla-only repopulation, and only in one thymus lobe. The medulla in these cases contains cells expressing medullary levels of Thy-1; cortical thymocytes always have about two to three times the amount of Thy-1 that medullary thymocytes have [12]. We have one case of cortex-only repopulation. The incidence of single foci does not change significantly for up to 2 months, and so it appears that a focus, once established, is self-renewing and unlikely to be displaced by another. These experiments show that it is technically feasible to look at clonogenic populations of cells that enter the thymus and reconstitute one region vs another [13]. Thus, it is now feasible to test in vivo at what stage and in which thymic microenvironment T cells gain their various commitments.

B. Receptor-Mediated Lymphogenesis

How is it possible that a retrovirus injected into a susceptible mouse strain will give rise to tumors that arise only in the thymus in a thymus-dependent fashion and after a latent period that can last up to 9 months after injection of the virus? One model that Baird, McGrath, and Weissman developed and tested was based on the receptor-mediated leukemogenesis hypothesis [14, 15]. In that model, these noncytopathic retroviruses selectively infect and transform the subset of those cells within the thymus that have antigen-specific (and/or growth factor) receptors directed against the virus. All clonal progeny of that particular infection and transformation would be cells having high levels of antigen-specific receptors directed against the envelope glycoproteins of the virus that induced them. Proliferation of normal T cells is usually dependent on two events. The first event is engagement of the T cell antigen-specific receptor with antigen, followed by transition of the cells from G_0 to G_1. At least one subset of T cells which engage antigen make a T cell growth factor, TCGF, now called interleukin 2 (IL-2). As a function of antigen recognition at least one other subset of T cells express the IL-2 growth factor receptor, and then proliferate. So proliferation of T lymphocytes depends on antigen recognition for all cell types, and IL-2 recognition by at least a subset of these cells. We proposed that retroviruses cause leukemias by using the normal mitogenic pathway, so that the virus which binds to that antigen-specific receptor can both infect the cell and cause it to undergo blastogenesis. Thus, the daughter cells would continually produce the same virus for which they have antigen-specific receptors, and triggering of these antigen-specific receptors would be an essential part of the mitogenic pathway. By cell-to-cell interaction or virus–cell interaction these daughter cells would be in a positive feedback loop of proliferation. This was (I think) the first autocrine hypothesis. Of course, it is entirely possible that the infected cell is an antigen-presenting cell, and in that context in vivo there would be a *microenvironment-dependent* malignant proliferation of lymphoid cells. We have performed several experiments to try to test (or rule out) the hypothesis, which cannot be explained by any of the cellular oncogene activation models alone (promoter-insertion-mediated oncogene activation, pX-mediated oncogene activation, or enhancer-mediated oncogene activation). More information on these experiments follows:

Is it possible that every T cell lymphoma in fact is a clone of cells having on their surface receptors with a high degree of specificity for the virus that induces the tumor? To test that prediction of the hypothesis we fluoresceinated (or rhodaminated) a number of leukemogenic retroviruses and used the FACS to assay the tumors for virus-binding receptors. Of over 40 T lymphomas examined, all bind their inducing retrovirus, and always with a high degree of specificity [15–17]. Although the high degree of specificity described here matches the diversity of antigen receptors, demonstration of specificity alone does not prove that the binding sites are the T cell receptors.

In a second set of experiments we wanted to determine the relevance of these kinds of receptors to lymphomagnesis in

vivo. To study this, we assayed the thymuses of AKR mice (which regularly get tumors somewhere between 6 and 12 months of age) for binding with these various retroviruses. Very few receptors could be found in either the neonatal or the preleukemic period. Receptor-positive thymuses could always transfer donor-derived leukemias to congenic AKR/Thy-1.2 mice, whereas receptor-negative thymuses could not. In some cases, during preleukemic thymic involution the residual cells in the thymus were 50%–85% virus-binding population. FACS sorting of virus-binding cells in these cases gave rise to donor-derived leukemias upon transfer; virus-nonbinding cells from the same thymuses failed to give rise to donor-derived leukemia [17]. Whatever these receptors are, they are a clear diagnostic sign for leukemia cells.

Is virus binding required for lymphoma cell proliferation? We raised a number of monoclonal antibodies directed against AKR lymphoma cell surface determinants to check for those that would block virus binding. Four antibodies were found that blocked virus binding. Three of the four were directed against the Thy-1 determinant. The four antibodies that block virus binding were also antiproliferative; up to 1 : 10 000 dilution of them blocked 90% of thymidine incorporation into AKR lymphoma cells, while antibodies to other cell surface determinants could not [18]. Presaturation of the surface of these cells with high concentrations of purified cognate retrovirus allowed the cells to proliferate in the presence of the blocking antibodies. Other viruses that bind less well to the cell surface do not protect against the antiproliferative effect. Antibody inhibition of proliferation has been shown with KKT-2 cells [18] and S49 cells [19]. It is difficult to explain this finding by any other retrovirus leukemogenesis hypothesis.

C. Are the Virus Receptors on Lymphoma Cells Antigen Receptors?

Allison was the first to produce a monoclonal antibody that identifies the antigen-specific T cell receptor heterodimer, using a particular T cell tumor called C6VL [20]. C6VL is a RadLV-induced tumor. The anti-T cell receptor antibody, Mab124-40, was a clonotypic antibody; it recognized a variable region determinant on the C6VL T cell receptor. Mab124-40 – C6VL T cell receptor immune complexes were used to raise a rabbit antibody, 8177, which recognizes T cell receptors on many different T cells [21]. To test C6VL-RadLV/C6VL interactions we developed a plate-binding assay in which a microtiter plate is precoated with dilutions of retrovirus and then cells are added for binding; the washed plates are poststained for cells with Rose Bengal. C6VL cells bind to RadLV/C6VL at high virus dilutions, to RadLV/VL3 slightly less well, and to KKT-2 SL (an AKR leukemia virus) only poorly. The clonotypic antibody Mab124-40 significantly blocks binding of the cells to the plate over a 30-fold dilution range, as does 8177. Antibodies to H-2D^b determinants on these cells do not block binding. These experiments indicate that virus receptors do exist on C6VL cells, and that the virus receptor is *at* or *near* the T cell antigen-specific receptors for this lymphoma.

We have strong evidence in two other systems that retrovirus binding is to an antigen-specific receptor. One of these systems is the B cell lymphoma, called BCL_1, described by Slavin and Strober [22]. BCL_1 is a B cell tumor which expresses high levels of IgM and low levels of IgD, and grows in animals as a spleen-dependent tumor [23, 24]. The in vivo BCL_1 tumor dies rapidly in vitro as a lymphoid cell suspension, but in vitro it persists in clusters with I-A$^+$ surface-adherent cells. The BCL_1 lymphocytes produce no retrovirus, but the adherent cells produce high levels of particles with the classical retrovirus polypeptide profile. BCL_1 X NS1 hybridomas produce and secrete the BCL_1 immunoglobulin. Plate-bound BCL_1 hybridoma immunoglobulin binds the retrovirus produced by those adherent cells, while other myeloma immunoglobulins of the same class do not. Anti-μ, anti-BCL_1 anti-idiotype, and even anti-gp70 antibodies block virus binding to this purified immunoglobulin. The anti-idiotype antibodies also block binding of the virus to the cell. Thus BCL_1 is one case in which the virus binding

entity is an immunoglobulin on the cell surface.

The best example of what appears to be insertion activation of cellular *myc* genes is the avian leukosis system. We expected that these avian leukosis virus-induced bursal lymphomas would not have immunoglobulins that bound ALV, but that is probably wrong. We obtained from J.M. Bishop a bursal tumor called SC2L, induced by RAV ALV. Labeled RAV virus binds well to SC2L cells, and added cold RAV blocks in the binding as expected. Isolated SC2L chicken immunoglobulin stuck to a plate binds labeled RAV, and that binding is blocked with cold RAV or with Max Cooper's monoclonal anti-chicken light chain antibodies. The same antibodies block RAV binding to SC2L cells, whereas anti-Ia antibodies do not (Ia is abundant on SC2L cells). The anti-light chain antibodies also block RAV binding to bursal lymphomas as they arise in vivo, using samples provided by G. Gasic and W. Hayward. The large bursal cells which are abundant in the preleukemic period do not bind RAV. If one believes that the virus binding shown has relevance to the leukemogenic process, these experiments show that virus binding does not drive preleukemic bursal cell proliferation. Thus, as in the AKR model, the appearance of cells bearing high levels of leukemogenic retrovirus receptors heralds the leukemic state.

Finally, in preliminary experiments we have done binding studies with HTLV. ATL cells bind it well, and this binding can be blocked with cold HTLV. Although we have evidence that monoclonal antibodies to a number of T cell determinants do not inhibit HTLV binding to ATL cells, we have not completed that analysis and do not yet have antibodies to the human T cell receptor heterodimer.

References

1. Weissman I (1967) Thymus cell migration. J Exp Med 126:291
2. Cantor H, Weissman IL (1976) Development and function of subpopulations of thymocytes and T lymphocytes. Prog Allergy 20:1
3. Jotereau FV, LeDouarin NM (1982) Demonstration of a cyclic renewal of lymphocyte precursor cells in the quail thymus during embryonic and perinatal life. J Immunol 129:1869
4. Weissman I, Papaioannou V, Gardner R (1978) Fetal hematopoietic origins of the adult hematolymphoid system. In: Clarkson B, Marks PA, Till JE (eds) Cold Spring Harbor Meeting on Differentiation of Normal and Neoplastic Hematopoietic Cells. pp 33–47
5. Scollay R, Butcher E, Weissman IL (1980) Thymus cell migration. Quantitative aspects of cellular traffic from the thymus to the periphery in mice. Eur J Immunol 10:210
6. Weissman I (1973) Thymus cell maturation: studies on the origin of cortisone-resistant thymic lymphocytes. J Exp Med 137:504
7. Scollay R, Shortman K (1983) Thymus 5:245
8. Butcher EC, Weissman IL (1984) Lymphoid tissues and organs. In: Paul WE (ed) Fundamental immunology. Raven, New York, pp 109–127
9. Gallatin WM, Weissman IL, Butcher EC (1983) A cell surface molecule involved in organ-specific homing of lymphocytes. Nature 304:30
10. Reichert RA, Gallatin WM, Butcher EC, Weissman IL (1984) A homing receptor-bearing cortical thymocyte subset: implications for thymus cell migration and the nature of cortisone-resistant thymocytes. Cell 38:89
11. Fink PJ, Gallatin WM, Reichert RA, Butcher EC, Weissman IL (1985) Homing receptor-bearing thymocytes: an immunocompetent cortical subpopulation. Nature 3/3:233
12. Fathman CG, Small M, Herzenberg LA, Weissman IL (1975) Thymus cell maturation. II: Differentiation of three "mature" subclasses in vivo. Cell Immunol 15:109
13. Ezine SV, Weissman IL, Rouse RV (1984) Bone marrow cells give rise to distinct cell clones within the thymus. Nature 309:629
14. Weissman IL, Baird S (1977) Oncornavirus leukemogenesis as a model for selective neoplastic transformation. In: Koprowski H (ed) Neoplastic transformation: mechanisms and consequences. (Life sciences research report no. 7)
15. McGrath MS, Weissman IL (1978) A receptor mediated model of viral leukemogenesis: hypothesis and experiments. In: Clarkson B, Marks PA, Till JE (eds) Cold Spring Harbor Meeting on Differentiation of Normal and Neoplastic Hematopoietic Cells. pp 577–589
16. McGrath MS, Pillemer E, Kooistra D, Weissman IL (1980) The role of MuLV receptors

on T lymphoma cells in lymphoma cell proliferation. Contemp Top Immunobiol 11: 257–284
17. McGrath MS, Weissman IL (1979) AKR leukemogenesis: identification and biological significance of thymic lymphoma receptors for AKR retroviruses. Cell 17:65
18. McGrath MS, Pillemer E, Weissman IL (1980) Murine leukemogenesis: monoclonal antibodies to cell determinants which arrest T lymphoma cell proliferation. Nature 285:259
19. McGrath MS, Jerabek L, Pillemer E, Steinberg RA, Weissman IL (1981) Receptor-mediated murine leukemogenesis: monoclonal antibody-induced lymphoma cell growth arrest. In: Neth R (ed) Modern trends in human leukemia, vol 4. Springer, Berlin Heidelberg New York (Haematology and blood transfusion, vol 26)
20. Allison JP, McIntyre BW, Bloch D (1982) J Immunol 129:2293
21. McIntyre BW, Allison JP (1983) Cell 34:739
22. Slavin S, Strober S (1977) Spontaneous murine B cell leukemia. Nature 272:624
23. Knapp MR, Jones PO, Black SJ, Vitetta ES, Slavin S, Strober S (1979) Characterization of the spontaneous murine B cell leukemia (BCL_1). I: Cell surface expression of IgM, IgD, Ia, and FCR. J Immunol 123:1000
24. Kotzin B, Strober S (1980) Role of the spleen in the growth of a B cell leukemia. Science 208:259