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B cells and B cell products-helping to restore cellular immunity?

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Summary

T cells that provide vital protection against tumors, viruses and intracellular bacteria are thought to develop independently of B cells. However, recent discoveries suggest that development of T cells depends on B cells. One way B cells promote T cell development is by providing diverse peptides that may promote positive selection of thymocytes. Diverse peptides and B cells help in diversification of the T cell receptor repertoire and may decrease cross-reactivity in the mature T cell compartment. These new insights may provide the basis for the design of novel therapeutics.

Keywords

B cells; T cell selection; Thymus; Cellular Immunity

Introduction

Perhaps the greatest advance in clinical immunology in the twentieth century was the development of immunosuppressive drugs. The development of immunosuppressive drugs allowed the enduring transplantation of organs and tissues and the amelioration and reversal of some autoimmune conditions. Today, the greatest challenge in clinical immunology is no longer the design of better strategies for immunosuppression (although that challenge still exists) but rather the development of approaches to expand immunity, particularly cell-mediated immunity (to some extent, humoral immunity can be replaced or expanded by the administration of gamma globulin or immune globulins of various types). Expanding of cell-mediated immunity could address the urgent problems posed by acquired immunodeficiency syndrome and the complications of cancer chemotherapy. Expanding cell-mediated immunity could also address a set of auto-immune conditions that arise paradoxically in those whose immune system has been attenuated by aging or by disease. Expanding of cell-mediated immunity might also help address some the challenges posed by the growing incidence of cancers.

While drugs such as 6-mercaptopurine developed to suppress proliferation of cancer cells could be directly applied to suppressing immunity; what agents or strategies might be applied to expanding immunity is less clear. Recent advances in understanding how T cells develop however may provide some clues that could be exploited toward this end.

T cells are thought to develop independently of B cells. Thus, T cells and B cells develop in two distinct anatomical places: T cells in the thymus, and B cells in the bone marrow. While T cells are produced predominantly in youth and their production declines progressively following adolescence, B cells are continuously made throughout life. The distinct anatomical

compartments and production kinetics have been interpreted as demonstrating independent lineage, hence the designation of “T” and “B” lineage cells [1].

Humans and mice deficient of T cells (e.g., DiGeorge syndrome) do have B cells [2], and humans and mice that are deficient of B cells do have T cells [3,4]. These simple observations suggest that at least some cells of each lineage can be produced independently of cells of the other lineage. However, the observations fail to explain why T cell and B cell functions are abnormal in individuals lacking the other cell type. We can now say that enumerating T cells and B cells is not enough to predict function, much less to conclude how each compartment developed. We shall discuss below how assumptions about development failed.

T cell development

T cells develop in the thymus, mostly in youth, and the thymus atrophies dramatically with age. In mice, T cells are generated exclusively after birth [5], while in humans T cell development starts in the fetal period [6]. In the thymus, immature T cells undergo a series of developmental steps. The progress of maturation is usually defined based on cell surface expression patterns of CD4 and CD8 receptors. The most primitive thymocytes do not express CD4 or CD8 and are hence designated as CD4⁻ CD8⁻ double-negative. As development of thymocytes proceeds, CD4 and CD8 are both expressed, and hence thymocytes at the next stage are said to be double-positive. Next, thymocytes lose expression of either CD4 or CD8, and hence they become positive for one or the other and are called “single positive” thymocytes [7].

Development not only yields a mature phenotype, it also assures that the mature cells can contribute to cell mediated immunity. T cells recognize foreign but not self-peptides embedded in MHC class I or II molecules. Hence, one result of T cell development must be the production of a “repertoire” of T cells that is diverse enough to anticipate a vast number of foreign peptides presented in the context of self-MHC but that at the same time are not auto-reactive. The complexity of this task can be appreciated if one considers; first, that assembly of the T cell receptor (TCR) genes generates molecules potentially capable of binding to any imaginable conformation of MHC and peptide; and second, the conformations of self-MHC that mature T cells are desired to recognize (self-MHC+ foreign peptide) are not present during development.

The generation of a functional T cell repertoire is achieved in the thymus by coupling random rearrangement of TCR gene segments to a stringent selection pressure, limiting the survival of nascent T cells to those that bind conformations of self-MHC and self-peptides not too well. T cells that bind self-MHC and peptide too well or too poorly die. The thymocytes selected to survive (positively selected) form the mature repertoire of T cells after they emerge from the thymus [8-11].

The diversity of the TCR repertoire and the probability that selected T cells can recognize and bind a given foreign peptide presented by self-MHC is thus directly related to the number of different self-MHC conformations that mediate selection. Given that the MHC shape is determined to a great extent by the bound peptide [12], peptide diversity determines the variety of MHC conformations available to select thymocytes. Thus, T cells that mature in mice expressing a single peptide associated with MHC class II are cross-reactive (less restricted) and more frequently alloreactive than T cells that mature in a normal environment with diverse peptides [8,13].

The nature and properties of the cells that present those peptides are important variables in the generation of T cell diversity by diverse peptides. In the thymus, there are various cells capable of presenting peptides, and for many years thymic epithelial cells were considered largely

responsible for this function [14]. However, it could be shown that thymocyte selection did not depend only on the thymic epithelium [15]. What other cells might present peptides to developing thymocytes has, until recently, been unknown. Our recent work suggests those other cells may include B cells.

There is reason to think that cells of bone marrow origin such as B cells might provide some of the MHC and/or peptide used by thymocytes during development. B cells comprise 1% of thymocytes [16,17] and are located mostly at the cortico-medullary junction [16,18] where selection takes place. Thymic B cells may participate in negative selection of thymocytes; thus, thymic B cells injected into thymi of neonatal mice promote deletion of auto-reactive thymocytes that recognize the B cells' MHC or endogenous murine mammary tumor viruses' superantigens [19,20] and also delete superantigen-reactive thymocytes *in vitro* [21]. However, B cells are not required for T cell negative selection because B cell-deficient mice delete superantigen reactive T cells [22].

While most B cells in the thymus are mature and have an activated phenotype [17,21,23], B cell progenitors can also be found [16,24,25]. While the proportion of B cells in the thymus remains constant from birth to 150 days in the mouse, the numbers of precursor B cells expressing the stem cell marker c-kit decreases with age more than 100-fold [26], suggesting a function for thymic B cell precursors in youth, perhaps contributing T cell development by means other than antigen presentation.

Monoclonal B cells counteract negative selection of transgenic T cells

To study whether and how B cells might interact with T cells, my colleagues and I developed strains of mutant mice with defined populations of B cells and T cells. Mice with monoclonal T cells expressing the DO 11.10 (DO) TCR (DO-T), which recognize a peptide of ovalbumin in the context of MHC class II^d, were bred to monoclonal B cell mice [27,28]. We studied the development of DO-T cells in mice with and in mice without monoclonal B cells, the monoclonal B-T and the monoclonal T mice, respectively [29]. In the monoclonal T mice, the DO-T cells are deleted in the thymus by binding to MHC class II^b presenting nominal endogenous peptides, and these have only rare conventional CD4⁺ T cells in the periphery. Contrastingly, monoclonal B-T mice had 36-fold more CD4⁺ T cells in the spleen and at least two-fold more CD4⁺ cells in the thymus than monoclonal T mice [29]. These findings suggested that the monoclonal B cells might have promoted T-cell development [18] by counteracting negative selection.

B cell diversity is required for the development of a diverse T cell repertoire

Our findings indicating that monoclonal B cells rescue T cells from negative selection suggested the possibility that B cells enhance survival of developing thymocytes [29] by promoting positive selection (Figure 1) [18]. We predicted that if B cells promote positive selection of thymocytes then both thymocyte numbers and the TCR repertoire diversity should be decreased in B cell-deficient mice. Consistent with that prediction, we found that B cell-deficient mice have reduced numbers of thymocytes affecting equally all stages of T cell development (CD4- and CD8-double negatives, CD4- and CD8-double positives, CD4-single positives and CD8-single positives). Because positive selection requires expression of a functional T cell receptor, our results showing reduction in the number of CD4- and CD8-double-negative T cells that lack functional T cell receptor indicates that B cells may influence the development of T cells prior to selection.

To test whether B cells also promote diversification of the T cell repertoire, we quantified TCR diversity, taking advantage of a novel method developed by Ogle *et al.* [30]. TCR diversity, primarily determined by V(D)J recombination [31], decreases progressively during

development in the thymus since immature thymocytes (as many as 95% of thymocytes) that fail to express TCR or that express a TCR that binds too strongly or too weakly to MHC die. Since death by neglect and failure of positive selection predominates over death due to negative selection [32], we reasoned that diversity of the T cell receptor repertoire directly reflects the efficiency of positive selection. Consistent with the possibility that B cells promote positive selection, we found that B cell-deficient mice have a million-fold contraction of the thymocyte repertoire compared with wild type mice [33]. Our findings indicating that B cells and/or their products promote diversification of the TCR repertoire did not exclude decreased negative selection as a possible mechanism.

B cells might promote positive selection by providing a source of diverse peptides. Peptides might be generated from the variable regions of immunoglobulin molecules or from proteins expressed in the periphery. These peptides might be captured by B cells and transported to the thymus, just as B cells transport some antigens to lymph nodes.

Because T-cell diversity in B cell-deficient mice is restored by administration of polyclonal immunoglobulin, it would seem that B cells promote the positive selection of T cells by providing diverse peptides that would not otherwise be found in the thymus [33]. B cells might also contribute to negative selection, although the absence of autoimmunity in B cell-deficient mice or in mice with oligoclonal B cells does not favor a critical role of B cells in negative selection.

T cell functions and B cell-dependent T cell repertoire diversification

Positive selection of peptide-restricted T cells may explain how B cells promote the functions of T cells. To what extent the function of T cells actually depends on the diversity of the TCR repertoire has been difficult to ascertain. Certainly the TCR repertoire is abnormal in acquired immunodeficiency [34]; however, the defects in T cell function could be caused by a lack of CD4⁺ T cells or functional defects in existing T cells rather than by a decreased repertoire. A primary decrease in the T cell repertoire with normal numbers of T cells predicts defective T cell priming in response to antigens that may be recognized by very infrequent T cells (1 in 5 × 10⁴ to 1 in 10⁵), such as minor histocompatibility antigens and protein antigens other than MHC [35]. Corroborating this idea, Linton and collaborators [36] found a decreased frequency of antigen-specific CD4⁺ T cells as early as 5 days after priming with keyhole limpet hemocyanin in B cell-deficient mice. On the other hand, one would expect that responses to allogeneic MHC, which can be recognized by up to 10% of T cells [37], should be relatively unaffected by B cell-deficiency and contracted T cell repertoires that such deficiency causes. Consistent with this prediction, the classical studies in B cell-deficient chickens reveal normal rates of rejection of MHC disparate grafts, even though delayed type of hypersensitivity responses are impaired [38].

B cell-dependent T cell positive selection could affect the functions of T cells independently of effects on the repertoire *per se*. B cells may enhance selection of restricted T cell clones relatively to more promiscuous clones. Thus, in situations where positive selection is compromised, by immunoglobulin oligoclonality for example, T cells may be more cross-reactive. Cross-reactive T cells predict relatively unimpaired T cell primary responses and increased frequency of alloreactive T cells. The predictions are consistent with our experimental observations showing that skin allografts are rejected by B cell oligoclonal recipient mice as fast as by wild type mice [39].

Conclusion

Loss of cell-mediated immunity poses one of the greatest challenges in modern medicine and therapeutics. The problems associated with acquired immunodeficiency syndrome, chronic

immunosuppression and cancer chemotherapy provide the most dramatic examples. However, loss of cell-mediated immunity with aging and with various auto-immune disease is the most prevalent. These problems impel those interested in clinical immunology to focus on strategies for building up immunity, especially cell-mediated immunity. Most efforts to enhance cell-mediated immunity have been directed at the development of improved adjuvants (chemicals that activate antigen-presenting cells) or antigenic substances. But if the problem stems from a limit in the number or specificity of T cells available to respond then the more effective strategy would be directed at increasing the production of new T cells. Toward that end, we have reviewed certain recent insights from our own work. This work suggests that B cells and B cell products might yield a new set of therapeutics to address the vexing challenge of defects in cell-mediated immunity.

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References

1. Roitt IM, Greaves MF, Torrigiani G, et al. The cellular basis of immunological responses. A synthesis of some current views. *Lancet* 1969;2:367–371. [PubMed: 4183985]
2. Cancrini C, Romiti ML, Finocchi A, et al. Post-natal ontogenesis of the T-cell receptor CD4 and CD8 Vbeta repertoire and immune function in children with DiGeorge syndrome. *J Clin Immunol* 2005;25:265–274. [PubMed: 15981092]
3. Chen J, Trounstein M, Alt FW, et al. Immunoglobulin gene rearrangement in B cell deficient mice generated by targeted deletion of the JH locus. *Int Immunol* 1993;5:647–656. [PubMed: 8347558]
4. Kitamura D, Roes J, Kuhn R, Rajewsky K. A B cell-deficient mouse by targeted disruption of the membrane exon of the immunoglobulin mu chain gene. *Nature* 1991;350:423–426. [PubMed: 1901381]
5. Miller JFAP. Effect of neonatal thymectomy on the immunological responsiveness of the mouse. *Proc Royal Soc London* 1962;156:415–428.
6. Spits H. Development of alphabeta T cells in the human thymus. *Nat Rev Immunol* 2002;2:760–772. [PubMed: 12360214]
7. Zuniga-Pflucker JC. T-cell development made simple. *Nat Rev Immunol* 2004;4:67–72. [PubMed: 14704769]
8. Ignatowicz L, Kappler J, Marrack P. The repertoire of T cells shaped by a single MHC/peptide ligand. *Cell* 1996;84:521–529. [PubMed: 8598039]
9. Surh CD, Lee DS, Fung-Leung WP, et al. Thymic selection by a single MHC/peptide ligand produces a semidiverse repertoire of CD4+ T cells. *Immunity* 1997;7:209–219. [PubMed: 9285406]
10. Tourne S, Miyazaki T, Oxenius A, et al. Selection of a broad repertoire of CD4+ T cells in H-2Ma0/0 mice. *Immunity* 1997;7:187–195. [PubMed: 9285404]
11. van Meerwijk JP, Marguerat S, Lees RK, et al. Quantitative impact of thymic clonal deletion on the T cell repertoire. *J Exp Med* 1997;185:377–383. [PubMed: 9053438]
12. Marrack P, Bender J, Jordan M, et al. Major histocompatibility complex proteins and TCRs: do they really go together like a horse and carriage? *J Immunol* 2001;167:617–621. [PubMed: 11441061]
13. Huseby ES, White J, Crawford F, et al. How the T cell repertoire becomes peptide and MHC specific. *Cell* 2005;122:247–260. [PubMed: 16051149]
14. Kyewski B, Derbinski J. Self-representation in the thymus: an extended view. *Nat Rev Immunol* 2004;4:688–698. [PubMed: 15343368]
15. Zinkernagel RM, Althage A. On the role of thymic epithelium vs. bone marrow-derived cells in repertoire selection of T cells. *Proc Natl Acad Sci USA* 1999;96:8092–8097. [PubMed: 10393953]
16. Akashi K, Richie LI, Miyamoto T, et al. B lymphopoiesis in the thymus. *J Immunol* 2000;164:5221–5226. [PubMed: 10799882]
17. Miyama-Inaba M, Kuma S, Inaba K, et al. Unusual phenotype of B cells in the thymus of normal mice. *J Exp Med* 1988;168:811–816. [PubMed: 3261779]

18. Cascalho M, Platt JL. B cell-dependent T cell development. *Acta Paediatr* 2004;93:52–53.
19. Inaba M, Inaba K, Hosono M, et al. Distinct mechanisms of neonatal tolerance induced by dendritic cells and thymic B cells. *J Exp Med* 1991;173:549–559. [PubMed: 1900075]
20. Kleindienst P, Chretien I, Winkler T, Brocker T. Functional comparison of thymic B cells and dendritic cells in vivo. *Blood* 2000;95:2610–2616. [PubMed: 10753841]
21. Ferrero I, Anjuere F, Martin P, et al. Functional and phenotypic analysis of thymic B cells: role in the induction of T cell negative selection. *Eur J Immunol* 1999;29:1598–1609. [PubMed: 10359114]
22. Beutner U, Kraus E, Kitamura D, et al. B cells are essential for murine mammary tumor virus transmission, but not for presentation of endogenous superantigens. *J Exp Med* 1994;179:1457–1466. [PubMed: 8163931]
23. Hayakawa K, Hardy RR. Development and function of B-1 cells. *Curr Opin Immunol* 2000;12:346–353. [PubMed: 10858035]
24. Mori S, Inaba M, Sugihara A, et al. Presence of B cell progenitors in the thymus. *J Immunol* 1997;158:4193–4199. [PubMed: 9126980]
25. McKenna HJ, Morrissey PJ. Flt3 ligand plus IL-7 supports the expansion of murine thymic B cell progenitors that can mature intrathymically. *J Immunol* 1998;160:4801–4809. [PubMed: 9590227]
26. Ceredig R. The ontogeny of B cells in the thymus of normal, CD3 epsilon knockout (KO), RAG-2 KO and IL-7 transgenic mice. *Int Immunol* 2002;14:87–99. [PubMed: 11751756]
27. Cascalho M, Ma A, Lee S, et al. A quasi-monoclonal mouse. *Science* 1996;272:1649–1652. [PubMed: 8658139]
28. Cascalho M, Martin DA, Wong J, et al. A mouse with a monoclonal primary immunoglobulin repertoire not further diversified by V-gene replacement. *Dev Immunol* 1999;7:43–50. [PubMed: 10636478]
29. Keshavarzi S, C. R, Simoes S, et al. The possibility of B-cell-dependent T-cell development. *Scand J Immunol* 2003;57:446–452. [PubMed: 12753501]
30. Ogle BM, Cascalho M, Joao CM, et al. Direct measurement of lymphocyte receptor diversity. *Nucleic Acids Res* 2003;31:e139. [PubMed: 14602932]
31. Nikolich-Zugich J, Slifka MK, Messaoudi I. The many important facets of T-cell repertoire diversity. *Nat Rev Immunol* 2004;4:123–132. [PubMed: 15040585]
32. Surh CD, Sprent J. T-cell apoptosis detected in situ during positive and negative selection in the thymus. *Nature* 1994;372:100–103. [PubMed: 7969401]
33. João CM, Ogle BM, Gay-Rubenstein C, et al. B cell-dependent TCR diversification. *J Immunol* 2004;172:4709–4716. [PubMed: 15067046]
34. Killian MS, Monteiro J, Matud J, et al. Persistent alterations in the T-cell repertoires of HIV-1-infected and at-risk uninfected men. *AIDS* 2004;18:161–170. [PubMed: 15075532]
35. Kanagawa O, Louis J, Cerottini JC. Frequency and cross-reactivity of cytolytic T lymphocyte precursors reacting against male alloantigens. *J Immunol* 1982;128:2362–2366. [PubMed: 6174631]
36. Linton P, Harbertson J, Bradley LM. A critical role for B cells in the development of memory CD4 cells. *J Immunol* 2000;165:5558–5565. [PubMed: 11067910]
37. Suchin EJ, Langmuir PB, Palmer E, et al. Quantifying the frequency of alloreactive T cells in vivo: new answers to an old question. *J Immunol* 2001;166:973–981. [PubMed: 11145675]
38. Szenberg A, Warner NL. Dissociation of immunological responsiveness in fowls with a hormonally arrested development of lymphoid tissues. *Nature* 1962;194:146–147. [PubMed: 13874945]
39. Abu Attieh MQ, Rebrovich ML, Platt JL, Cascalho M. Cellular immunity in B cell oligoclonal mice. In Preparation

Figure 1.

B Cells and Thymocyte Selection. T cell development starts with seeding of the thymus at the cortico-medullary junction by hematopoietic precursors. Early T cell-committed cells lack expression of T cell receptor (TCR), CD4 and CD8 and are denominated double-negative (DN) thymocytes. As they mature, DN thymocytes migrate toward the cortex and assemble the TCR genes. Thymocytes that fail to produce functional TCR die by neglect. TCR expression drives proliferation and expression of CD4 and CD8 markers originating double-positive (DP) thymocytes. Whether DP thymocytes mature into CD4 or CD8-single positive (SP) cells depends on the interaction affinity of the TCR with MHC plus endogenous peptide. Thymocytes expressing a TCR that binds to MHC plus self-peptide with intermediate affinity survive, i.e., are positively selected, whereas if the interaction is too weak or too strong they undergo death by neglect or negative selection, respectively. Positive selection of thymocytes has been generally attributed to thymic epithelial cells. However, our own work suggests that B cells (B) may also promote positive selection. B cells were shown to promote negative selection to viral superantigens (see text).