HHS Public Access

Author manuscript

J Immunol. Author manuscript; available in PMC 2017 February 13.

Published in final edited form as:

J Immunol. 2015 October 01; 195(7): 2923–2925. doi:10.4049/jimmunol.1501804.

Bone marrow cell rejection, MHC, NK cells and missing self recognition: Ain't that peculiar (with apologies to those too young to know of Marvin Gaye)

David H. Raulet

Department of Molecular and Cell Biology, University of California, Berkeley, CA 94720

Much modern research is quite focused, attempting to understand aspects of systems that are already revealed in broad outline. Researching a problem at its birth is an opportunity that many will never experience, a lonely exercise that many may avoid. In these situations, pre-existing findings from other scientists offer only limited help. Years or even decades may be necessary to make real progress, and, most discouragingly, the initial surprising findings may be of limited interest to scientists focused on more established systems or problems. Working on such a novel problem has its perks: witnessing an unexpected new biological system come to light is uniquely exciting, even if the emergence is gradual. The Pillars papers by Cudkowicz and Bennett reviewed here (1, 2) represent a good example of such emergent research. They focused on a phenomenon mediated by cells (NK cells) that had not yet been discovered, and that use a recognition strategy that had not yet been conceived. As a graduate student in the late 1970s, I was introduced to these papers by my supervisor, Michael Bevan, and found them intriguing. However, it is fair to say that the studies were of middling interest to most immunologists at that time, probably because they made little sense within the prevailing paradigms. Dr. Cudkowicz died young at age 54 in 1982, well before his phenomenon was understood, but Dr. Bennett lived to see, and indeed contribute to, the evolution of his findings into a basic understanding of how MHC molecules inhibit NK cell functions, before he died recently in January, 2015.

Before discussing the papers in detail, some background will be helpful. NK cells discriminate abnormal cells from normal ones using several recognition strategies. One recognition mechanism is called “missing self” recognition and is mediated by inhibitory receptors that are specific for MHC class I molecules, and are displayed on the plasma membranes of NK cells. Normal cells expressing MHC I engage these inhibitory receptors and prevent lysis of the normal cells by NK cells, whereas infected or transformed cells often suppress MHC I expression (presumably to evade CD8 T-cell recognition), and thus are more susceptible to being killed by NK cells. The first hints of missing self recognition came in a roundabout way from the discovery of a phenomenon called hybrid resistance. First described in the 1960s (3, 4), these findings culminated in 1971 in the two Pillars papers discussed here, from Cudkowicz and Bennett, with the unforgettable titles, “Peculiar

Pillars papers featured: Cudkowicz, G., and M. Bennett. 1971. Peculiar immunobiology of bone marrow allografts. I. Graft rejection by irradiated responder mice. *J. Exp. Med.* 134: 83-102.

Cudkowicz, G., and M. Bennett. 1971. Peculiar immunobiology of bone marrow allografts. II. Rejection of parental grafts by resistant F1 hybrid mice. *J. Exp. Med.* 134: 1513-1528.

Immunobiology of Bone Marrow Allografts. I and II" (1, 2). The results were surprising and well ahead of their time. NK cells were only discovered a few years later (5, 6), and several more years passed before it was realized that NK cells mediate this peculiar immunobiology. The molecular basis of this recognition process was only solved in the 1990s.

The role of the MHC in rapid rejection of skin or solid organ transplants was well studied by the 1960s. The pattern of rejection between strains with different MHC alleles led to the "laws of transplantation", which are in accord with the familiar notion that tissue with foreign MHC is rejected: MHC homozygotes (for illustration, $MHC^{a/a}$) reject allogeneic ($MHC^{b/b}$) or heterozygous ($MHC^{a/b}$) tissue, whereas $MHC^{a/b}$ heterozygotes accept tissue from either homozygous parent ($MHC^{a/a}$ or $MHC^{b/b}$). Cudkowicz et al. examined irradiated mice that were injected with bone marrow from various strains. They used a specialized assay to detect proliferating donor bone marrow cells as an index of successful engraftment. As with other types of transplants, $MHC^{a/a}$ mice rejected $MHC^{b/b}$ bone marrow, at least in some combinations (allorejection). Unlike other tissue types, however, $MHC^{a/a}$ bone marrow transplants were rejected by $MHC^{a/b}$ heterozygous (F1 hybrid) mice, which led to the sobriquet "F1 hybrid resistance" to describe the phenomenon (3). This pattern of bone marrow cell rejection violated the known laws of transplantation.

Hybrid resistance was first reported in earlier papers by Cudkowicz and Stimpfling (3, 4), as was the fact that rejection of fully allogeneic bone marrow also unexpectedly occurs in irradiated mice. However, the two 1971 papers featured in this commentary represented the most comprehensive analysis of the phenomena while highlighting exactly how unusual these responses were. Peculiar I examined rejection of fully allogeneic cells, whereas Peculiar II examined rejection of parental strain bone marrow by F1 hybrids, that is, hybrid resistance. Later studies showed that allorejection of bone marrow is especially complicated, because both T cells and NK cells may contribute to rejection, apparently even in irradiated hosts at late time points (7). Nevertheless, we now know that rejection of allogeneic bone marrow by NK cells is mediated in part by the same mechanisms as hybrid resistance. This is because the donor marrow cells lack MHC molecules of the recipient, just as donor $MHC^{a/a}$ parental marrow cells lack MHC molecules of an $MHC^{a/b}$ F1 recipient.

Aside from the unusual genetics of bone marrow rejection, another oddity was that it occurred in heavily irradiated mice, in which other familiar immune reactions are usually defective. A third feature, documented in Peculiar II, is that it occurs within 2 days of bone marrow inoculation, whereas T and B cell reactions generally take longer to fully develop in naïve animals. A fourth distinctive feature is that it is restricted to lymphohematopoietic cell grafts, because skin grafts and other solid tissues from $MHC^{a/a}$ mice are accepted by $MHC^{a/b}$ mice. These unusual features were the bases of the designation "peculiar" in the titles of the two featured papers.

Evidence emerged between 1977 and 1987 that bone marrow rejection and hybrid resistance are primarily mediated by NK cells and not T cells, as had been generally assumed (7-9). The peculiar features of hybrid resistance can be explained by differences between T cells and NK cells. NK cells are resistant in the short-term to irradiation, but they eventually die off after 5-10 days. Accordingly, bone marrow allograft rejection (as described in Peculiar I)

and hybrid resistance (as described in Peculiar II) occur when marrow cells are injected immediately after initial irradiation, whereas rejection fails when the marrow graft is delayed for 5-10 days, even when the animals are re-irradiated. The very rapid rejection of marrow grafts can be explained by the presence of appreciable numbers of NK cells in normal animals, whereas T cell responses require activation and expansion of a smaller population of Ag-specific cells to become maximally functional. The reason that hybrid resistance is restricted to lymphohematopoietic cells remains unclear to this day, but likely reflects the selective expression on those cells of ligands that engage distinct NK cell activation receptors (10).

The most peculiar and interesting aspect of bone marrow rejection by NK cells was the recognition mechanism, enigmatically hinted at by the underlying genetics of hybrid resistance: how can NK cells in $MHC^{a/b}$ animals recognize and reject $MHC^{a/a}$ cells? The resolution of this question helped elucidate the missing self principle of NK cell recognition, later proposed by Ljunggren and Karre (11). It was known that conventional MHC molecules are expressed in a codominant fashion, so $MHC^{a/b}$ animals should be tolerant of MHC^{a} molecules on $MHC^{a/a}$ cells. One possibility discussed in Peculiar II was that the relevant Ags were not conventional MHC molecules, but were distinct Ags, termed “hybrid histocompatibility” (Hh) Ags, encoded by genes closely linked to MHC that are only expressed in homozygotes (2). This view became untenable years later when it was shown that expression of a conventional MHC transgene (D^{d}) was sufficient to enable $H\text{-}2^{b/b}$ mice to reject $H\text{-}2^{b/b}$ bone marrow cells (12). It appeared that the absence of a host's conventional MHC I molecules was the feature targeted by NK cells. Previously, Karre et al. (13) had shown that tumor cells with low MHC I are targeted by NK cells, and my group subsequently showed that bone marrow cells from gene-targeted mice lacking all MHC I molecules are strongly rejected by NK cells in normal mice (14). Thus, NK cells reject bone marrow cells that lack one or more MHC molecules of the host.

The next big puzzle was how NK cells perceive missing MHC molecules. One notion was that MHC I molecules specifically block ligands on target cells that, when unmasked by loss of MHC, activate NK cell cytotoxicity. The alternative proposal, which turned out to be correct, was that MHC I molecules engage inhibitory receptors on NK cells that suppress NK cell cytotoxicity. Inhibitory receptors specific for MHC I were subsequently discovered in mice (15) and humans (16-19). These and later studies demonstrated that the action of these receptors underlies missing self recognition and hybrid resistance (15, 20, 21).

It still remained puzzling how the absence of only one parental MHC allele could be sufficient to render cells sensitive to NK cells in a heterozygous recipient. MHC molecules are expressed codominantly, so cells that lose expression of one particular MHC I molecule will still display others, which might be expected to continue to inhibit NK cells. A series of studies, some carried out by Bennett in cooperation with his long-time collaborator Vinay Kumar, helped reveal the answer: there are several distinct MHC I specific receptors, which discriminate allelic variants of MHC I. Moreover, these receptors are displayed on different, although overlapping, sets of NK cells. Hence, some NK cells in the MHC heterozygote express inhibitory receptors that bind MHC^{a} and not MHC^{b}, and hence can kill $MHC^{b/b}$ cells, but not self ($MHC^{a/b}$) cells. Others express inhibitory receptors that bind MHC^{b} and

not MHC^{a}, and hence kill MHC$^{a/a}$ cells (20). NK cells that lack both types of receptors also exist; however, those NK cells are likely disabled in an MHC$^{a/b}$ mouse, given evidence that MHC$^{a/a}$ mice contain NK cells that lack receptors for MHC^{a}, and these NK cells are functionally impaired (22, 23).

The revelations arising from the study of hybrid resistance and missing self recognition by NK cells have been employed to increase the success and therapeutic benefits of bone marrow transplantation for leukemia patients. Abundant evidence indicates that including mature donor immune cells in a bone marrow graft is beneficial in several respects. In the case of bone marrow grafts in humans or mice in which the recipient lacked MHC Ags of the donor, the presence of donor NK cells in the graft resulted in decreased leukemia burden in the hosts, improved engraftment and reduced graft versus host disease (24). These outcomes were attributed to action of NK cells killing host cells lacking donor MHC molecules, including host leukemia cells, residual host lymphohematopoietic cells (thus facilitating engraftment), and host antigen presenting cells (thus inhibiting induction of donor T cell responses against host cells, that is, graft-versus-host disease). More generally, the discovery of hybrid resistance was pioneering, as it may represent the first glimmering of inhibitory recognition by immune cells. Inhibitory recognition is now a well-established feature of recognition by NK cells, B cells, T cells, and myeloid cells. Indeed, it is a significant basis for checkpoint immunotherapies, which are revolutionizing the clinical management of cancer.

References

1. Cudkowicz G, Bennett M. Peculiar immunobiology of bone marrow allografts. I. Graft rejection by irradiated responder mice. J Exp Med. 1971; 134:83–102. [PubMed: 4397663]
2. Cudkowicz G, Bennett M. Peculiar immunobiology of bone marrow allografts. II. Rejection of parental grafts by resistant F1 hybrid mice. J Exp Med. 1971; 134:1513–1528. [PubMed: 4942407]
3. Cudkowicz G, Stimpfling JH. Deficient growth of C57BL marrow cells transplanted into F1 hybrid mice. Immunology. 1964; 7:291–306. [PubMed: 14169112]
4. Cudkowicz G, Stimpfling JH. Induction of immunity and of unresponsiveness to parental marrow grafts in adult F1 hybrid mice. Nature. 1964; 204:450–453. [PubMed: 14232531]
5. Kiessling R, Klein E, Wigzell H. “Natural” killer cells in the mouse. I. Cytotoxic cells with specificity for mouse Moloney leukemia cells. Specificity and distribution according to genotype. Eur J Immunol. 1975; 5:112–117. [PubMed: 1234049]
6. Herberman RB, Nunn ME, Lavrin DH. Natural cytotoxic reactivity of mouse lymphoid cells against syngeneic and allogeneic tumors. I. Distribution of reactivity and specificity. International Journal of Cancer. 1975; 16:216–229. [PubMed: 50294]
7. Murphy WJ, Kumar V, Bennett M. Acute rejection of murine bone marrow allografts by natural killer cells and T cells. J Exp Med. 1987; 166:1499–1509. [PubMed: 3316472]
8. Kiessling R, Hochman PS, Haller O, Shearer GM, Wigzell H, Cudkowicz G. Evidence for a similar or common mechanisms for natural killer cell activity and resistance to hemopoietic grafts. Eur J Immunol. 1977; 7:655–663. [PubMed: 336380]
9. Murphy W, Kumar V, Bennett M. Rejection of bone marrow allografts by mice with severe combined immunodeficiency (SCID). J Exp Med. 1987; 165:1212–1217. [PubMed: 3549961]
10. Dong Z, Cruz-Munoz ME, Zhong MC, Chen R, Latour S, Veillette A. Essential function for SAP family adaptors in the surveillance of hematopoietic cells by natural killer cells. Nat Immunol. 2009; 10:973–980. [PubMed: 19648922]
11. Ljunggren HG, Karre K. In search of the ‘missing self’: MHC molecules and NK cell recognition. Immunol Today. 1990; 11:237–244. [PubMed: 2201309]

12. Ohlen C, Kling G, Höglund P, Hansson M, Scangos G, Bieberich C, Jay G, Karre K. Prevention of allogeneic bone marrow graft rejection by H-2 transgene in donor mice. Science. 1989; 246:666–668. [PubMed: 2814488]

13. Karre K, Ljunggren HG, Piontek G, Kiessling R. Selective rejection of H-2-deficient lymphoma variants suggests alternative immune defense strategy. Nature. 1986; 319:675–678. [PubMed: 3951539]

14. Bix M, Liao NS, Zijlstra M, Loring J, Jaenisch R, Raulet D. Rejection of class I MHC-deficient haemopoietic cells by irradiated MHC-matched mice. Nature. 1991; 349:329–331. [PubMed: 1987491]

15. Karlhofer FM, Ribaudo RK, Yokoyama WM. MHC class I alloantigen specificity of Ly-49+ IL-2 activated natural killer cells. Nature. 1992; 358:66–70. [PubMed: 1614533]

16. Moretta A, Vitale M, Borrino C, Orengo A, Morelli L, Augugliaro R, Barbaresi M, Ciccone E, Moretta L. P58 molecules as putative receptors for major histocompatibility complex (MHC) class I molecules in human natural killer (NK) cells. Anti-p58 antibodies reconstitute lysis of MHC class I-protected cells in NK clones displaying different specifities. J Exp Med. 1993; 178:597–604. [PubMed: 8340759]

17. Colonna M, Samaridis J. Cloning of immunoglobulin-superfamily members associated with HLA-C and HLA-B recognition by human natural killer cells. Science. 1995; 268:405–408. [PubMed: 7716543]

18. Wagtmann N, Biassoni R, Cantoni C, Verdiani S, Malnati M, Vitale M, Bottino C, Moretta L, Moretta A, Long E. Molecular clones of the p58 natural killer cell receptor reveal Ig-related molecules with diversity in both the extra- and intra-cellular domains. Immunity. 1995; 2:439–449. [PubMed: 7749980]

19. Lanier LL. NK Cell Recognition. Annu Rev Immunol. 2004

20. Yu YY, George T, Dorfman J, Roland J, Kumar V, Bennett M. The role of Ly49A and 5E6 (Ly49C) molecules in hybrid resistance mediated by murine natural killer cells against normal T cell blasts. Immunity. 1996; 4:67–76. [PubMed: 8574853]

21. Held W, Cado D, Raulet DH. Transgenic expression of the Ly49A natural killer cell receptor confers class I major histocompatibility complex (MHC)-specific inhibition and prevents bone marrow allograft rejection. J Exp Med. 1996; 184:2037–2041. [PubMed: 8920892]

22. Fernandez NC, Treiner E, Vance RE, Jamieson AM, Lemieux S, Raulet DH. A subset of natural killer cells achieves self-tolerance without expressing inhibitory receptors specific for self-MHC molecules. Blood. 2005; 105:4416–4423. [PubMed: 15728129]

23. Shifrin N, Raulet DH, Ardolino M. NK cell self tolerance, responsiveness and missing self recognition. Semin Immunol. 2014; 26:138–144. [PubMed: 24629893]

24. Ruggeri L, Capanni M, Urbani E, Perruccio K, Shlomchik WD, Tosti A, Posati S, Rogaia D, Frassoni F, Aversa F, Martelli MF, Velardi A. Effectiveness of donor natural killer cell alloreactivity in mismatched hematopoietic transplants. Science. 2002; 295:2097–2100. [PubMed: 11896281]