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## Bone Marrow Cell Rejection, MHC, NK Cells, and Missing Self Recognition: Ain't That Peculiar (with Apologies to Marvin Gaye)

David H. Raulet

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. 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 (MHC 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<sup>+</sup> 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 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<sup>a/a</sup>) reject allogeneic (MHC<sup>b/b</sup>) or heterozygous (MHC<sup>a/b</sup>) tissue, whereas MHC<sup>a/b</sup> heterozygotes accept tissue from either homozygous parent (MHC<sup>a/a</sup> or MHC<sup>b/b</sup>). 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<sup>a/a</sup> mice rejected MHC<sup>b/b</sup> bone marrow, at least in some combinations (allorejection). Unlike other tissue types, however, MHC<sup>a/a</sup> bone marrow transplants were rejected by MHC<sup>a/b</sup> heterozygous (F<sub>1</sub> hybrid) mice, which led to the sobriquet “F<sub>1</sub> 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 F<sub>1</sub> 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

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donor MHC<sup>a/a</sup> parental marrow cells lack MHC molecules of an MHC<sup>a/b</sup> F<sub>1</sub> 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 d of bone marrow inoculation, whereas T and B cell reactions generally take longer to fully develop in naive animals. A fourth distinctive feature is that it is restricted to lymphohematopoietic cell grafts, because skin grafts and other solid tissues from MHC<sup>a/a</sup> mice are accepted by MHC<sup>a/b</sup> 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 d. 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 d, even when the animals are reirradiated. 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<sup>a/b</sup> animals recognize and reject MHC<sup>a/a</sup> cells? The resolution of this question helped elucidate the missing self principle of NK cell recognition, later proposed by Ljunggren and Kärre (11). It was known that conventional MHC molecules are expressed in a codominant fashion, so MHC<sup>a/b</sup> animals should be tolerant of MHC<sup>a</sup> molecules on MHC<sup>a/a</sup> cells. One possibility discussed in Peculiar II was that the relevant Ags were not conventional MHC molecules, but were distinct Ags, termed “hybrid histocompatibility” 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<sup>d</sup>) was sufficient to enable H-2<sup>b/b</sup> mice to reject H-2<sup>b/b</sup> 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, Kärre 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<sup>a</sup> and not MHC<sup>b</sup>, and hence can kill MHC<sup>b/b</sup> cells, but not self (MHC<sup>a/b</sup>) cells. Others express inhibitory receptors that bind MHC<sup>b</sup> and not MHC<sup>a</sup>, and hence kill MHC<sup>a/a</sup> cells (20). NK cells that lack both types of receptors also exist; however, those NK cells are likely disabled in an MHC<sup>a/b</sup> mouse, given evidence that MHC<sup>a/a</sup> mice contain NK cells that lack receptors for MHC<sup>a</sup>, 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 APCs (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 basis for checkpoint immunotherapies, which are revolutionizing the clinical management of cancer.

## Disclosures

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