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Innate Allorecognition by Monocytic Cells and its Role in Graft Rejection

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Abstract

Innate recognition of microbial products and danger molecules by monocytes and macrophages has been well established; this is mediated primarily by pattern recognition receptors and is central to activation of innate and adaptive immune cells required for productive immunity. Whether monocytes and macrophages are equipped with an allorecognition system that allows them to directly respond to allogeneic grafts is a topic of much debate. Recent studies provide compelling evidence that these cells are capable of recognizing allogeneic entities and mediate graft rejection via direct cytotoxicity and priming of alloreactive T cells. These studies have also uncovered a mechanism of innate allorecognition based on detection of the polymorphic molecule SIRPα on donor cells. Further understanding of innate allorecognition and its consequences would provide essential insights into allograft rejection and lead to better therapies for transplant patients.

Transplantation of cells, tissues, or organs between genetically-distinct individuals poses a persistent challenge to the host immune system. Ischemia reperfusion injury of the graft, surgical trauma to the host, release of danger molecules by stressed cells, exposure to microbial products, as well as the burden of major and minor histoincompatibility antigens are powerful stimulators of the recipient's immune and inflammatory systems (1). It is therefore not surprising that graft rejection involves multiplicity of immune cells, including innate and adaptive cells, which have been difficult to fully control in clinical transplantation.

At the center of graft rejection is the recognition of allogeneic antigens (allorecognition) by the immune system (2). The rejection process is dependent on T lymphocytes, the cardinal cells of the adaptive immune system. The principal alloantigens detected by T lymphocytes are the polymorphic major histocompatibility complex (MHC) molecules widely expressed on bodily tissues. T cell receptors (TCR) recognize amino acid polymorphisms in MHC molecules and/or in the peptides bound to them, placing the MHC-TCR interaction at the center of the canonical allorecognition process. This MHC-TCR interaction also defines the donor specificity and memory features of the rejection response. Because of this, clinical

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interventions aimed at preventing transplant rejection are mostly focused on the adaptive T cells.

Despite the persistence of innate immune cells in grafts long after the immediate post-transplantation period, the question whether they themselves detect allogeneic antigens has remained unanswered until recently. Emerging studies in animal models have provided compelling evidence that innate cells, including those of the monocytic lineage (monocytes and macrophages), engage in allorecognition (3). This form of non-microbial, non-self recognition, referred to here as *innate allorecognition*, plays an important role in transplant rejection for two fundamental reasons. First, it provides a means by which host monocyte-derived, mature antigen-presenting dendritic cells (DCs) are continually induced after transplantation – cells that we now know are essential for initiating as well as sustaining the anti-donor T cell response (4, 5). Second, it contributes to graft destruction by inducing delayed type hypersensitivity (DTH)-like innate responses and macrophage allocytotoxicity. Both events are detrimental to grafts. Here we will focus on monocytes/macrophages, summarizing recent evidence that establishes the existence of innate allorecognition in the mouse and highlight its roles in T cell activation, allocytotoxicity, and graft rejection. We will describe a recently identified mechanism by which these cells detect allogeneic grafts. We will also briefly touch throughout this review upon an intriguing feature of the innate alloresponse: innate memory.

It is not all about danger

The conventional thinking has been that the stimuli responsible for DC maturation, the first step in triggering the alloimmune response, derive from danger molecules released by stressed or dying cells in the immediate period after organ transplantation. This line of thought has led to a plethora of investigations that identified many ligands and receptors that cause or potentiate DC activation, but have failed to identify a principal pathway that when interrupted prevents rejection in stringent models (6, 7). It also fell short of explaining the paradox that resolution of danger over time does not guarantee that allografts become invisible to the immune system – they are invariably rejected when T cell number or function is restored (8).

The possibility that, in addition to danger, non-self allogeneic antigens trigger DC maturation and/or induce other forms of innate reactions did not emerge until investigators began to carefully interrogate the innate immune responses of immunodeficient mice to allogeneic *versus* syngeneic grafts. In 2001, Fox et al reported that intraperitoneal injection of xenogeneic tumor cells into *SCID* mice, which lack T cells and B cells, elicited significantly greater monocyte and neutrophil recruitment than the injection of an equal number of syngeneic tumor cells (9), suggesting that the innate immune system not only responds to danger signals but also to non-self xenodeterminants. Incidentally, allogeneic tumor cells also caused somewhat greater innate cell recruitment than syngeneic cells but the statistical significance of this difference was not determined, neither was the contribution of NK cells to the xenogeneic or allogeneic responses (9). Several years later, Zecher et al provided direct evidence that the mouse innate immune system does indeed distinguish between self and non-self allogeneic antigens independently of adaptive immune cells (10).

They demonstrated that subcutaneous injection of allogeneic splenocytes from lymphocyte-deficient $RAG^{-/-}$ donors elicited a DTH-like reaction in $RAG^{-/-}$ recipients, while syngeneic cells did not. Depletion and cell transfer experiments established that the response was not mediated by NK cells but by monocytes. Of note, this innate alloresponse was most conspicuous if mice were previously primed with donor cells one, or even four, weeks earlier, suggesting that this type of allorecognition is manifested in both primary and memory responses. A subsequent study by Liu et al showed that, after an initial priming phase, macrophages acquire the ability to identify and kill allogeneic cells independent of the concomitant presence of adaptive lymphoid cells (11). Unlike the Zecher experiment, however, $CD4^+$ T cells were required for preparing macrophages to become allocytotoxic and this occurred via a CD40-dependent pathway. In this model $CD4^+$ T cells upregulates CD40L when challenged by alloantigens, which then engages CD40 on alloantigen-stimulated macrophages to render them allospecific in their toxicity (11). Therefore, the innate monocytes and macrophages, have or acquire the ability to sense allogeneic antigens, leading to DTH-like pathology or direct killing of target cells. Moreover, they exhibit a memory-like feature since they mount an anamnestic reaction to previously encountered alloantigens. This memory feature is not well understood, but in other models enhanced macrophage responses to pathogens after previous encounters with microbial products are related to epigenetic modifications of certain genomic loci (12). The innate memory following microbial pathogen encounters, also called trained immunity, responds to broad microbial products in recall responses, thus lacking antigen specificity. In contrast, the monocyte/macrophage memory we have reported clearly exhibits alloantigen specificity, highlighting fundamental differences between these two model systems in the induction of innate memory.

Perhaps the most persuasive evidence so far for the existence of innate allorecognition, independent of known forms of allorecognition by adaptive immune cells, is the demonstration by Oberbarnscheidt et al that allografts transplanted between $RAG^{-/-}\gamma c^{-/-}$ mice, which lack T, B, NK, and innate lymphoid cells, are rapidly infiltrated with host monocyte-derived DCs (mono-DCs) that have a mature phenotype, produce IL-12, and persist well beyond the immediate post-transplantation danger period (13). In this model, no prior priming of recipients with donor cells was needed to elicit the monocyte alloresponse, although priming did lead to allospecific memory that lasted up to seven weeks after immunization. In contrast, syngeneic grafts transplanted to the same type of recipients behaved differently. They were transiently infiltrated with a significantly smaller number of mono-DCs that were less mature and, above all, did not produce IL-12. Immunization with syngeneic cells did not enhance subsequent monocyte responses. The observation that innate allorecognition generates DCs from monocytes has been confirmed since by another group. Chow et al showed that intravenous transfer of allogeneic but not syngeneic leukocytes causes rapid accumulation of host mono-DCs in mice without any increase in conventional DCs (14). Collectively, these data establish that the innate monocyte response to allogeneic grafts is quantitatively and qualitatively distinct from that to syngeneic grafts despite the commonality of danger signals associated with the grafting procedure.

What does it all have to do with graft rejection?

Analogous to microbial infection (15), activation of DCs by allogeneic grafts links innate to adaptive immunity. In the experiments reported by Oberbarnscheidt et al (13), mono-DCs isolated from allografts proved to be potent antigen-presenting cells that, by virtue of IL-12 production, drove both T cell proliferation and IFNγ production. In contrast, mono-DCs derived from syngeneic grafts induced T cell proliferation but the T cells did not produce IFNγ. Additional experiments showed that *in vivo* exposure of monocytes to allogeneic antigens precipitated T cell-mediated rejection of single minor Ag-mismatched heart grafts that are otherwise accepted by the host and, conversely, depletion of mono-DCs blunted rejection significantly (13). Therefore, in this model the allogeneic antigens have adjuvant properties to monocytes similar to pathogen associated molecular patterns such as LPS, while danger or inflammation alone (as is the case with the transplantation of a syngeneic graft) is not sufficient for inducing the Th1 immune response typically associated with graft rejection. Inability of inflammatory mediators alone to fully activate DCs to induce a Th1 response has been previously shown in a model other than transplantation (16). In addition, macrophages, once becoming allospecific, may directly contribute to acute and chronic graft damage by acting as potent cytolytic cells (10).

IL-12-producing DCs generated from monocytes also propagate the alloimmune response within the graft by forming cognate interactions with effector and memory T cells. These interactions enhance transmigration and retention of effector/memory T cells and lead to their increased survival and proliferation (5). In addition, macrophages, once becoming allospecific, may directly contribute to acute and chronic graft damage by acting as potent cytolytic cells (11). Therefore innate allorecognition can potentially trigger or enhance graft rejection by (a) generating mature DCs that activae naïve and effector/memory T cells and (b) inducing allotoxic activity in macrophages.

A molecular mechanism of innate allorecognition by monocytes

Significant progress in identifying the molecular mechanisms that underlie innate allorecognition has been made. A recently completed genetic mapping study in the mouse demonstrated that recipient monocytes detect polymorphism in donor signal regulatory protein alpha (SIRPα) that influences the binding of SIRPα to its receptor CD47 on host monocytes (17). SIRPα is highly expressed on myeloid cells and delivers inhibitory signals that suppress such cells. The ligand for SIRPα is the ubiquitously expressed molecule CD47 (18), and dual signaling that is either inhibitory (via SIRPα) or stimulatory (via CD47) regulates monocyte, DC and macrophage functions, guaranteeing tolerance to self in the innate immune system when the two signaling pathways are in balance. However, the introduction of an allograft with a SIRPα molecule that is mismatched with that of the recipient creates an imbalance that in some cases - if donor SIRPα has higher affinity to CD47 than self SIRPα - causes monocytic cell activation (17). In other situations, such as in certain tumor models, SIRPα signaling promotes the generation of myeloid derived suppressor cells which inhibit anti-tumor immunity (19). Conversely, blocking engagement of SIRPa by CD47 promotes tumor elimination by enhancing DC function, including the cross-priming of anti-tumor CD8 T cells (20, 21). Therefore, the distinction between self

and allogeneic non-self in the innate immune system appears to be mediated by a balance between inhibitory and stimulatory molecules, some of which are polymorphic, which allows them to function as allodeterminants. It is possible that molecules other than SIRPα, which are still to be determined, are also involved in allorecognition by monocytic cells.

Unanswered questions

Three unresolved questions related to the nascent field of monocyte/macrophage allorecognition come to mind. First is the question whether additional molecular mechanisms responsible for the primary and memory innate alloresponses and whether or how they are linked to each other. Studies towards uncovering the molecular identity of such ligands and receptors are fundamental for moving this area of research forward and for developing novel therapeutic interventions. Second is whether innate allorecognition contributes to graft rejection in experimental settings that resemble clinical transplantation; for example, transplantation of MHC-mismatched or multiple minor histocompatibility-mismatched grafts to immunocompetent recipients. This is an important area of future investigation, especially in relation to chronic rejection where monocytes/macrophages are clearly dominant. Considering that chronic rejection remains the most common cause of graft loss despite conventional immunosuppression, which primarily targets adaptive allorecognition, the prospect of targeting innate allorecognition could potentially help combat chronic rejection. Third, whether human monocytic cells show the same features of alloreactivity, including reliance on sensing donor SIRPα polymorphism, deserves careful investigation. In fact, DCs and macrophages are present in significant numbers in chronically rejected transplants in humans, and the degree to which they are present correlates with poor kidney allograft outcomes (22).

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Abbreviations

DC	dendritic cell
DTH	delayed type hypersensitivity
MHC	major histocompatibility complex
TCR	T cell receptor

References

1. Chong AS, Alegre ML. The impact of infection and tissue damage in solid-organ transplantation. Nat Rev Immunol. 2012; 12(6):459–471. [PubMed: 22627862]
2. Lakkis FG, Lechler RI. Origin and biology of the allogeneic response. Cold Spring Harbor perspectives in medicine. 2013; 3(8):pii–a014993.
3. Oberbarnscheidt MH, Lakkis FG. Innate allorecognition. Immunol Rev. 2014; 258(1):145–149. [PubMed: 24517431]

4. Liu Q, Rojas-Canales DM, Divito SJ, Shufesky WJ, Stolz DB, Erdos G, et al. Donor dendritic cell-derived exosomes promote allograft-targeting immune response. J Clin Invest. 2016; 126:2805–2820. [PubMed: 27348586]
5. Zhuang Q, Liu Q, Divito SJ, Zeng Q, Yatim KM, Hughes AD, et al. Graft-infiltrating host dendritic cells play a key role in organ transplant rejection. Nature Communications. 2016; 7 Article number: 12623.
6. Tesar BM, Zhang J, Li Q, Goldstein DR. TH1 immune responses to fully MHC mismatched allografts are diminished in the absence of MyD88, a toll-like receptor signal adaptor protein. Am J Transplant. 2004; 4(9):1429–1439. [PubMed: 15307830]
7. McKay D, Shigeoka A, Rubinstein M, Surh C, Sprent J. Simultaneous deletion of MyD88 and Trif delays major histocompatibility and minor antigen mismatch allograft rejection. Eur J Immunol. 2006; 36(8):1994–2002. [PubMed: 16874736]
8. Bingaman AW, Ha J, Waitze S-Y, Durham MM, Cho HR, Tucker-Burden C, et al. Vigorous allograft rejection in the absence of danger. J Immunol. 2000; 164:3065–3071. [PubMed: 10706695]
9. Fox A, Mountford J, Braakhuis A, Harrison LC. Innate and adaptive immune responses to nonvascular xenografts: evidence that macrophages are direct effectors of xenograft rejection. J Immunol. 2001; 166(3):2133–2140. [PubMed: 11160265]
10. Zecher D, van Rooijen N, Rothstein D, Shlomchik W, Lakkis F. An innate response to allogeneic nonself mediated by monocytes. J Immunol. 2009; 183:7810–7816. [PubMed: 19923456]
11. Liu W, Xiao X, Demirci G, Madsen J, Li XC. Innate NK cells and macrophages recognize and reject allogeneic nonself in vivo via different mechanisms. J Immunol. 2012; 188(6):2703–2711. [PubMed: 22327074]
12. Saeed S, Quintin J, Kerstens HH, Rao NA, Aghajanirefah A, Matarese F, et al. Epigenetic programming of monocyte-to-macrophage differentiation and trained innate immunity. Science. 2014; 345(6204):1251086. [PubMed: 25258085]
13. Oberbarnscheidt MH, Zeng Q, Li Q, Dai H, Williams AL, Shlomchik WD, et al. Non-self recognition by monocytes initiates allograft rejection. J Clin Invest. 2014; 124(8):3579–3589. [PubMed: 24983319]
14. Chow KV, Delconte RB, Huntington ND, Tarlinton DM, Sutherland RM, Zhan Y, et al. Innate Allorecognition Results in Rapid Accumulation of Monocyte-Derived Dendritic Cells. J Immunol. 2016; 197(5):2000–2008. [PubMed: 27474076]
15. Iwasaki A, Medzhitov R. Regulation of adaptive immunity by the innate immune system. Science. 2010; 327(5963):291–295. [PubMed: 20075244]
16. Sporri R, Reis e Sousa C. Inflammatory mediators are insufficient for full dendritic cell activation and promote expansion of CD4+ T cell populations lacking helper function. Nat Immunol. 2005; 6(2):163–170. [PubMed: 15654341]
17. Dai H, Friday AJ, Abou-Daya KI, Williams AL, Mortin-Toth S, Nicotra ML, et al. Donor SIRPa polymorphism modulates the innate immune response to allogeneic grafts. Science Immunology. 2017; 2(12):eaam6202. [PubMed: 28783664]
18. Barclay AN, van den Berg TK. The interaction between signal regulatory protein alpha (SIRPa) and CD47: structure, function, and therapeutic target. Annu Rev Immunol. 2014; 32:25–50. [PubMed: 24215318]
19. Engblom C, Pfirschke C, Pittet MJ. The role of myeloid cells in cancer therapies. Nat Rev Cancer. 2016; 16(7):447–462. [PubMed: 27339708]
20. Tseng D, Volkmer JP, Willingham SB, Contreras-Trujillo H, Fathman JW, Fernhoff NB, et al. Anti-CD47 antibody-mediated phagocytosis of cancer by macrophages primes an effective antitumor T-cell response. Proc Natl Acad Sci U S A. 2013; 110(27):11103–11108. [PubMed: 23690610]
21. Liu X, Pu Y, Cron K, Deng L, Kline J, Frazier WA, et al. CD47 blockade triggers T cell-mediated destruction of immunogenic tumors. Nat Med. 2015; 21(10):1209–1215. [PubMed: 26322579]
22. Batal I, De Serres SA, Safa K, Bijol V, Ueno T, Onozato ML, et al. Dendritic Cells in Kidney Transplant Biopsy Samples Are Associated with T Cell Infiltration and Poor Allograft Survival. J Am Soc Nephrol. 2015; 26(12):3102–3113. [PubMed: 25855773]