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Cross-presentation of IgG-containing immune complexes

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Abstract

IgG is a molecule that functionally combines facets of both innate and adaptive immunity and therefore bridges both arms of the immune system. On the one hand, IgG is created by adaptive immune cells, but can be generated by B cells independently of T cell help. On the other hand, once secreted, IgG can rapidly deliver antigens into intracellular processing pathways, which enable efficient priming of T cell responses towards epitopes from the cognate antigen initially bound by the IgG. While this process has long been known to participate in CD4⁺ T cell activation, IgG-mediated delivery of exogenous antigens into a major histocompatibility complex (MHC) class I processing pathway has received less attention. The coordinated engagement of IgG with IgG receptors expressed on the cell-surface (FcγR) and within the endolysosomal system (FcRn) is a highly potent means to deliver antigen into processing pathways that promote cross-presentation of MHC class I and presentation of MHC class II-restricted epitopes within the same dendritic cell. This review focuses on the mechanisms by which IgG-containing immune complexes mediate such cross-presentation and the implications that this understanding has for manipulation of immune-mediated diseases that depend upon or are due to the activities of CD8⁺ T cells.

Keywords

Fcγ receptor; FcRn; Cross-presentation; IgG; Immune complex; Dendritic cells

Introduction

Multicellular organisms continuously encounter potential pathogens and damaged cells and have thus developed a multilayered defense arsenal to maintain physiological homeostasis. Integration of the various branches of this immunological defense system is critical for efficient and optimal protection against invasive threats. The initiation point for much of this cross-talk between the humoral and cellular components of innate and adaptive immunity is the manner in which the perturbation is first sensed, which has an important role in determining the direction of the ensuing immune response. Typically, recognition of an immunological target is mediated by receptors which either bind to conserved sequences localized on the cell surface, such as pattern recognition receptors (PRR) specific for pathogen-associated molecular patterns (PAMPs) or damage-associated molecular patterns (DAMPs), or to unique sequences contained within secreted molecules such as complement or immunoglobulins (Ig), which in the latter case can be secreted or expressed on the cell surface [1]. Immunoglobulins, including IgG, are unique in that they may be produced and function in both the innate and adaptive arenas of the immune response. Specifically, IgG can be produced by B cells via both T cell-dependent and -independent mechanisms, the latter of which allows for IgG participation in immediate defense as well as in the initial orchestration of an immune response through a variety of processes [2, 3]. For example, the interaction of IgG with a ligand is commonly associated with neutralization or targeting for antibody-dependent cell-mediated cytotoxicity (ADCC) [4]. However, it is being increasingly recognized that Ig, particularly of the abundant IgG iso-type, are potent integrators of innate and cellular immunity by virtue of their ability to deliver opsonized antigen(s) to antigen presenting cells (APC) lacking antigen specific receptors, thereby enabling the priming of T cell responses towards cognate antigen.

Monomeric IgG, either unbound by antigen or bound to a single antigenic epitope, are efficiently recycled out of most cells, including epithelial, endothelial, and B cells [5–8]. This process of exocytosis, which has also indirectly been shown for APC such as dendritic cells (DC), accounts for the long half-life of IgG in circulation [9, 10]. In contrast, multivalent IgG-opsonized antigens are able to bind to low-affinity IgG receptors on APC via their Fc region resulting in cross-linking of the receptors and subsequent internalization of the antigens. Once internalized, such extracellular antigens are typically routed to intracellular compartments capable of processing and presenting nominal peptides from the antigens in the context of major histocompatibility complex (MHC) class II molecules, which are displayed on the cell surface for interactions with CD4⁺ T cells [11]. Such IgG-binding Fc γ receptor (Fc γ R)-mediated endocytosis, processing, and presentation are an important and effective means of promoting T cell activation to extracellular antigens [11, 12]. In turn, these responses are important in the protection against extracellular pathogens through the elicitation of CD4⁺ T cell help for the generation of antibodies with high affinity and avidity [13–15]. Presentation of epitopes from such exogenously acquired antigen on MHC class I molecules, however, also occurs in a process known as cross-presentation which, when first described, countered the long-held dogma that MHC class I could only be loaded with endogenously acquired antigen [16–19]. Such cross-presentation allows the APC to effectively prime CD8⁺ T cells to antigens associated with pathogens that target intracellular locales and which need such effector functions for their cytolytic removal. As such, a single APC can simultaneously cross-present antigens via MHC class I to CD8⁺ T cells and present antigens via MHC class II to CD4⁺ T cells which provide the T cell help necessary for initiating CD8⁺ T cell effector responses [20–22]. This elegant system allows for a unique geographic localization of both pathways for optimal efficacy. While a variety of APC are capable of presenting exogenously derived antigens on MHC class II molecules, only DC have so far been recognized to efficiently cross-present antigens in vivo [23, 24]. In large part, this has been attributed to their specialized intracellular processing machinery yet

the sensitivity of the process can be greatly enhanced by targeted delivery of antigen to specific membrane receptors such as DEC-205 [25–27]. The presence of members of the Fc γ R family on the surface of most DC in addition to the expression of an intracellular routing IgG receptor, the neonatal Fc receptor for IgG (FcRn), within DC generates a natural system for highly efficient delivery of antigen into a cross-presentation pathway capable of promoting defense against intracellular pathogens and tumor cells while also contributing to the balance between tolerance and autoimmunity [28, 29]. This review will focus on recent insights into the mechanisms of cross-presentation when initiated by IgG-containing immune complexes and IgG-opsonized antigens and the pathophysiological importance of this process.

Cross-presentation of soluble antigens

We will introduce this topic by first considering DC and the manner in which this subset of cells cross-present non-opsonized (or soluble) antigens as a basis for understanding the cross-presentation of IgG-associated antigens. DC are a very diverse population of cells consisting of a variety of different subsets, the frequency of which varies according to both tissue localization and context [30, 31]. Two broad populations of DC can be described, conventional CD11c⁺ DC, which originate from a myeloid precursor, and plasmacytoid DC (pDC), which originate from a lymphoid precursor [32]. Conventional DC can further be subdivided into CD8⁺CD11b[−] and CD8[−]CD11b⁺ subsets within lymphatic tissue and CD103⁺CXCR3[−]CD8⁺CD11b[−] and CD103[−]CXCR3⁺CD8[−]CD11b⁺ subsets within tissues [32–35]. Under inflammatory conditions, tissues are also commonly invaded by a population of monocyte-derived CD8[−]CD11b⁺ DC which arise from circulating monocytes yet are capable of DC functions [36]. Critically, while considerable plasticity exists within DC populations, it is likely that the complement of cell-surface and intracellular machinery exhibited by a particular DC at any point in time will dictate its functional capacity [30, 32].

It is thus not surprising that DC subsets are heterogeneous in their ability to cross-present soluble antigens. In mice, it is largely the CD8⁺ or CD103⁺ subsets of DC which drive this process whereas in humans, cross-presenting DC are CD141⁺ (BDCA3⁺) [37–39]. Although recent studies have also documented the ability of other DC subsets to drive cross-presentation of soluble antigen in vitro, in the absence of additional stimuli, as discussed below, the efficiency with which these other DC subsets have been found to activate CD8⁺ T cells is consistently inferior to that of CD8⁺ DC [40, 41]. Thus, while a role for other DC types in driving cross-presentation of soluble antigen cannot be ignored, particularly in in vivo settings where considerable cross-talk is likely to occur between cell populations at different stages of their development, the extreme potency of CD8⁺ DC to conduct this process has been the prime focus of cross-presentation research to date. To this effect, while the mode of antigen delivery to a DC can modulate the efficiency of cross-presentation, it is not specialized antigen capture which endows the CD8⁺ DC lineage with superior cross-presenting capabilities for soluble antigen [40, 42]. Rather, their specific phenotype is associated with a specialized intracellular machinery that allows these cells to exhibit slow antigen degradation that results in the prolonged persistence of relatively intact antigen and the resulting preservation of epitopes conducive to loading onto MHC class I molecules [43–45]. A key element of this behavior is the manner in which the endosomes of such DC subsets undergo acidification. The endosomal compartments of typical phagocytes such as macrophages and neutrophils acidify to pH <5.0, which corresponds well with the optimal proteolytic activity of many lysosomal proteases located within these compartments [46]. While this permits highly efficient neutralization of potentially harmful phagocytic cargo and the generation of antigens for loading onto MHC class II molecules, it is generally believed that this also destroys epitopes capable of being loaded into the MHC class I groove, thereby precluding efficient cross-presentation by these cells. In contrast, the pH

within endosomes and phagosomes of immature CD8⁺ DC, which excel at the cross-presentation of soluble antigens, remains stabilized in a neutral range (pH 7.0–7.5) [47]. This can be attributed to several processes, including the inefficient assembly of the vacuolar ATPase (V-ATPase) within these compartments, the delayed maturation kinetics of the phagosomes and the increased recruitment of ER proteins, the latter two of which have both been attributed to the actions of Sec22b [48–50]. Concomitantly, recruitment of an active NADPH oxidase, NOX2, to the phagosomal membrane within these DC leads to the generation of reactive oxygen species (ROS); a process which actively consumes protons and thereby generates a sustained alkalinizing effect which is required for maintaining the compartmental pH near neutral [43]. NOX2 is delivered to phagosomes within these cells by fusion of “lysosome-related organelles” which are recruited in a Rab27a- and Rac2-dependent manner [44, 51]. While this process also delivers lysosomal proteins such as lysosome-associated membrane protein (LAMP) to the phagosomes, the continuous recruitment and action of NOX2 is sufficient to buffer the pH within these compartments and thus prevent the acidification seen in phagosomes of other phagocytes.

Details of how antigens are processed within this specialized cross-presentation pathway within CD8⁺ DC continue to emerge. Early studies have indicated that at least two major routes, dubbed the vacuolar or cytosolic pathways, exist by which the antigen may be processed [24]. The pathway which predominates likely depends upon the nature of the antigen and the mode of delivery into the cell. Soluble antigen in the vacuolar pathway is degraded in situ within the endosome or phagosome by resident proteases and the resulting peptides are then loaded directly onto empty endosomal MHC class I within the compartment [52–55]. This process is independent of both the transporter associated with antigen processing (TAP) and proteasomal degradation and depends on specific proteases such as cathepsin S [53, 56]. In contrast, antigen within the cytosolic cross-presentation pathway is first exported into the cytosol from the initial endosome or phagosome and, subsequent to ubiquitination, is proteasomally processed [57–59]. The peptides generated by this process are then either reimported into cross-presentation competent phagosomes or endosomes, in which they undergo further processing by the insulin regulated aminopeptidase (IRAP), or are transported back to the endoplasmic reticulum (ER) at which point they can enter into the classical MHC class I loading pathway involving the related ER aminopeptidase (ERAP) [56, 60–62]. The latter conclusion has been based upon the TAP dependency often observed for this process and the known residence of TAP in the ER. Increasing evidence, however, indicates that fusion between the ER and various other intracellular compartments is not uncommon and that TAP might indeed be present in the phagosomes, thereby supporting early conclusions that phagosomes may be able to function as self-sufficient cross-presentation bodies [19, 57, 58].

Controversy persists as to which, if either, of these pathways predominates and under what circumstances an antigen is preferentially directed towards one mechanism or another. An important caveat likely lying at the heart of this dispute is that these studies have utilized not only a variety of DC in which to conduct mechanistic investigations but also a diverse array of antigens. In particular, most mechanistic studies have limited themselves to studying cross-presentation in the context of soluble antigens delivered in the absence of any co-stimulatory or maturation inducing signal. Given that the latter signals are known to drastically alter the microenvironment within internalizing endosomes or phagosomes, it is critical to consider such variables when attempting to generalize conclusions from a single study [48]. This point is made very salient by recent evidence demonstrating that plasmacytoid DC (pDC), which very inefficiently cross-present soluble antigen, are potent cross-presenters when the antigen is delivered in conjunction with a costimulatory signal such as CpG [63–67]. When considering the bulk of studies performed to date in which cross-presentation has been studied in the context of secondary co-stimulation, particularly

that of PRR engagement by a microbial ligand, a clearer consensus emerges from the data. For example, in CD8⁺ DC isolated from mice suffering from mycobacterial peritonitis, IRAP is required for their cross-presentation function whereas it is dispensable for cross-presentation in steady-state CD8⁺ DC [68]. Furthermore, the vast majority of studies published to date having examined in vivo cross-presentation of bacterial (*Salmonella typhimurium*, *Listeria monocytogenes*, *Escherichia coli*), viral (Epstein-Barr virus, cytomegalovirus) or protozoan (*Toxoplasma gondii*) antigen sources have identified proteasomal degradation as a critical step for the efficient induction of CD8⁺ T cell activation by DC [57, 69–75]. While not excluding a role for vacuolar proteases, these data imply that the antigen is preferentially shuttled into the cytosolic cross-presentation pathway when delivered in the context of an additional immunological stimulus which induces DC activation or maturation and thereby alters the intracellular environment. The precise mechanism behind this remains unknown in spite of the fact that a large proportion of in vivo antigenic stimuli are complex and likely to trigger such modulatory innate PRR during their uptake or subsequent processing.

Receptor contribution to cross-presentation

One of the first natural PRR recognized to play a role in cross-presentation of soluble antigen was the mannose receptor (MR), which is a C-type lectin receptor expressed both on the cell surface and intracellularly in a variety of cell types including macrophages and DC [76, 77]. This collagen-sensitive receptor mediates the uptake of soluble antigens by DC and directs this antigen into the early endosomal system, thereby segregating the antigen away from harshly degradative lysosomal compartments and promoting cross-presentation of the antigen [77, 78]. Furthermore, this receptor has been linked to cytosolic translocation of imported soluble antigen in a manner consistent with that seen for antigens delivered in the context of other secondary stimuli, as discussed above [79].

The greatly enhanced ability of MR-internalized antigen to elicit effective cross-presentation has not gone unnoticed by those seeking to target therapeutic molecules into this pathway [80]. Conjugates of antigen, such as the cancer-testis antigen NY-ESO-1, and MR-specific monoclonal antibodies have been shown to greatly improve cross-presentation of the desired antigen above that of the soluble protein alone [81]. Furthermore, this conjugate was shown to stimulate the activation of NY-ESO-1-specific CD8⁺ T cells, a process known as cross-priming, as well as of CD4⁺ T cells. Similar efficacies in driving specific CD8⁺ T cell responses were seen when NY-ESO-1 was fused to a monoclonal antibody specific for another receptor, DEC-205, which is also highly expressed on CD8⁺ DC [81–84]. Indeed, additional groups have successfully targeted antigen into a cross-presentation pathway using fusions with DEC-205-specific monoclonal antibodies [82, 85]. The success of this strategy has so far been attributed entirely to the ability of the antibodies to target the selected extracellular receptor. However, these fusions were constructed either by conjugating whole IgG with the desired protein or by including the protein as a genetically engineered in-frame fusion with the IgG heavy chain and, as such, the Fc region in the therapeutic molecule remains intact [81, 82, 85]. Thus, the interaction of the Fc region in these constructs with additional receptors capable of IgG binding by the DC cannot be entirely excluded when explaining their mode of action. Indeed, it is notable that the first receptors ever demonstrated to potentiate cross-presentation of antigen by DC were in fact Fc γ R [29, 86–88].

The extended IgG binding receptor family

The family of receptors which bind IgG was originally limited strictly to Fc γ R, which are expressed on the surface of a wide variety of hematopoietic cells. This family includes both

activating and inhibitory Fc receptors and thus forms a tunable system capable of finely controlling the effects of IgG, the abundant levels of which in the blood can play a significant role in driving immune responses. Activating Fc γ R function largely through the action of an immunoreceptor tyrosine-based activating motif (ITAM) which, in most cases, is located not in the alpha chain of the receptors themselves but rather in a co-associating dimeric adaptor molecule which, in APC, is known as the common γ -chain [89, 90]. Following ligand-induced phosphorylation of the ITAMs in the γ -chain dimer by SRC family kinases, the protein tyrosine kinase SYK is recruited via its two SH2 domains and then autophosphorylated, initiating a complex downstream signaling cascade [91–94]. The activating family of Fc γ R includes Fc γ RI, Fc γ RIII and Fc γ RIV in mice [90]. The human family is more complex, including several variants of Fc γ RI as well as additional activating Fc γ RIIa and Fc γ RIIc receptors, both of which possess an ITAM in their cytoplasmic tail and are thus capable of signaling independently of the common γ -chain [95–99]. In contrast, the inhibitory Fc γ R in both humans and mice is Fc γ RIIb, in which a single immunoreceptor tyrosine-based inhibitory motif (ITIM) is located in the cytoplasmic tail. Phosphorylation of this ITIM leads to the recruitment of phosphatases, particularly SHIP-1, which subsequently mediates the immunosuppressive effects caused by Fc γ RIIb ligation [100]. An additional layer of complexity in the Fc γ R system is that various gain-of-function and loss-of-function polymorphisms for most of these receptors have been described, some of which are known to alter either ligand binding or downstream signaling [101–103].

While the downstream signaling pathways initiated by ligand binding are remarkably similar between the different activating Fc γ R, ligand binding affinity varies widely depending on the IgG subclass as well the valency of the ligand. Fc γ RI has a very high affinity for monomeric human IgG1 and, considering that this subclass is the most abundant in the serum, this receptor is typically saturated with ligand under physiological conditions [104]. In contrast, Fc γ RII and Fc γ RIII bind with low affinity to monomeric IgG of any subclass but bind extremely strongly to multimeric IgG within immune complexes (IC). Binding of such multivalent ligands cross-links the receptors and potently initiates the signaling cascades described above. The relative strength of the signal is largely determined by the balance between the frequency of activating and inhibitory Fc γ R which are triggered, as well as by the valence of the complex and nature of the ligand to which the IgG is bound.

The family of IgG binding receptors was expanded beyond Fc γ R upon the discovery of an additional IgG binding receptor, FcRn, which is an MHC class I-like molecule requiring interaction with β 2-microglobulin for proper folding and functioning [105–107]. While expression of this receptor was initially described only in the intestinal epithelial cells of neonatal rodents where it functions in the passive acquisition of IgG from the mother's milk, it has since been shown to be expressed throughout life not only in adult human intestinal epithelial cells but also in epithelial cells of the placenta, lung and liver as well as in endothelial cells and the majority of hematopoietic cells in humans and rodents [108, 109]. Although transcytosis of maternal IgG across the placenta, in humans, or across the intestinal epithelium of suckling pups, in rodents, were the first functions ascribed to FcRn, this molecule is now known to play a critical role in the maintenance of circulating IgG levels as well as in mucosal and systemic immune regulation [9, 28, 110–115]. Importantly, the binding site for FcRn on the Fc region of IgG is distinct from that where Fc γ R binds and FcRn–IgG binding is independent of glycosylation at amino acid Asn297 which is critical for Fc γ R–IgG interactions [116]. The wide variety of functions carried out by FcRn can largely be attributed to a combination of its predominantly intracellular distribution and its highly specialized binding characteristics. Specifically, FcRn binds IgG specifically at pH 6.0 such that binding is minimal at the neutral pH of the cell surface but strong in the acidic environment of the endocytic and phagocytic compartments, thereby enabling FcRn to act as an intracellular receptor and transporter for IgG [117]. While it is known that the short

cytoplasmic domain of FcRn contains both tryptophan- and dileucine-based basolateral targeting signals, two endocytosis signals (one each based on the combination of Trp311 with Leu314 and Leu322/Leu323 with Asp317/Asp318) and two serine phosphorylation sites (Ser313 and Ser319), the contribution, if any, of these domains to FcRn's function in hematopoietic cells, particularly DC, remains unknown [118–120].

The diversity of receptors having evolved with IgG binding function as well as their distribution across a variety of cellular compartments and their relative evolutionary conservation across species clearly demonstrates the importance of both extracellular and intracellular IgG transport to organismal functioning. Although the first demonstration of IgG-driven cross-presentation by Fc γ R occurred many years ago, characterization of the mechanisms involved including possible cooperativity between the different IgG receptors and the physiological consequences of the process have only recently begun to be elucidated. Critically, the importance of cross-presentation in the context of concomitant signaling by IgG for the activation of specific CD8⁺ T cell responses is increasingly being recognized [121].

The contribution of cell-surface Fc γ R to cross-presentation of IgG-complexed antigens

IgG binding by Fc γ R was first recognized to greatly increase the sensitivity of MHC class II presentation of exogenously derived IgG-complexed antigens and skew the repertoire of peptide epitopes generated for presentation [11, 12]. This was shown to depend upon signaling by the common γ -chain of activating Fc γ R but to be inhibited by ligation of the inhibitory Fc γ RIIb [11]. Shortly after this discovery, it was demonstrated that Fc γ R within immature DC also efficiently deliver antigens into an intracellular pathway conducive to cross-presentation [87]. Critically, delivery of antigen in this manner induced the concomitant maturation of the DC, as is typical for antigen delivery via a PRR, rendering it refractory to the cross-presentation of subsequent antigens contained within other IC likely by permanently altering the endolysosomal environment [48]. Cross-presentation of antigen delivered as an IC was shown in this study and a follow-up study to be dependent upon the common γ -chain and to depend upon both proteasomal processing of the antigen and peptide transport by TAP1–TAP2 [87, 88]. While DC were highly efficient at transferring IC-delivered antigen into the cytosol and inducing subsequent CD8⁺ T cell activation, similarly treated macrophages, which also express high levels of Fc γ R, retained protein within vesicles expressing high levels of the acid protease, cathepsin D, and failed to prime CD8⁺ T cells. This finding is supported by the observation that ligation of identical Fc γ R in DC and macrophages leads to differential intracellular signaling [122, 123]. Thus, while Fc γ R-binding by IgG IC is critical for initial antigen uptake, it is not sufficient to drive cross-presentation in the absence of an intracellular environment that is conducive to appropriate antigen degradation.

Nonetheless, the ability of Fc γ R to efficiently capture and internalize IgG-complexed antigens exerts potent effects on the ability of a given cell to cross-present. This point was emphasized by a study looking specifically at *in vivo* cross-presentation of IgG-complexed antigen by CD8⁺ or CD8[−] DC subsets expressing equivalent levels of Fc γ R. While the former were found to cross-present IC-delivered ovalbumin (OVA) in either the presence or absence of functional Fc γ R, consistent with the known facility of this DC subset to cross-present soluble antigen, loss of Fc γ R function via deletion of the common γ -chain abrogated the ability of CD8[−] DC to cross-present IgG-complexed OVA [124]. Perhaps even more convincing is that transfection of a non-professional APC epithelial cell coexpressing H2-K^b with Fc γ RIIa, which is capable of signaling in the absence of the common γ -chain, successfully conferred cross-presenting ability upon these cells when the

antigen was delivered as an IgG IC [97, 125]. Indeed, the initial study describing Fc γ R-mediated cross-presentation demonstrated that antigen delivered as an IgG IC was cross-presented with 3–4 orders of magnitude greater efficiency than the same antigen delivered in soluble form [87]. However, it is not simply the presence of Fc γ R on APC subsets but rather the relative proportion of activating versus inhibitory Fc γ R that may alter the cross-presenting capacity of the cell. While it is abundantly clear that engagement of the inhibitory Fc γ RIIb receptor by IgG IC is inhibitory towards cross-presentation and that an overabundance of this receptor in pDC may account for their poor capacity to cross-present, the relative contribution of the various activating Fc γ R remains controversial [126–128]. While some groups have shown in vivo that equivalent levels of CD8⁺ T cell activation are achieved upon administration of IgG-complexed OVA in WT, Fc γ RI/Fc γ RIII^{-/-} and Fc γ RI/Fc γ RII/Fc γ RIII^{-/-} mice, other groups have demonstrated that ligation of Fc γ RIII with multimeric IC leads to more efficient cross-presentation than ligation of Fc γ RI [121, 129]. The latter is perhaps not surprising given that different intracellular signaling cascades have been documented upon IgG IC ligation of Fc γ RI versus Fc γ RIII and that these affect both the trafficking of downstream mediators through the endosomal compartments and the differential induction of cytokine secretion [130]. While the details of how each Fc γ R contributes to cross-presentation are complex, as outlined in Table 1, the emerging consensus is that ligation of activating Fc γ R by IgG-complexed antigen and the subsequent downstream activation of SYK are critical steps to enabling cross-presentation in some cell types and to boosting the cross-presentation capacity of other cell types [131].

An important consideration raised by this knowledge is the fact that a wide variety of factors known to modulate surface expression levels or function of Fc γ R in DC will also affect the ability of these cells to cross-present complexed antigens. Whereas IL-4 is known to increase expression of the inhibitory Fc γ RIIb receptor and down-regulate expression of activating Fc γ R, exposure of cells to IFN γ has the opposite effect [132]. Exposure of monocytes and macrophages to low doses of galectin-1, a carbohydrate-binding protein, not only increases expression of Fc γ RI but also increases the rate of Fc γ RI-dependent phagocytosis by inducing the phosphorylation of ERK1/2 [133]. Interaction of periplakin with the cytoplasmic domain of Fc γ RI has also been shown to increase the ability of this Fc γ R to bind to and endocytose IgG-complexed antigen and also to increase the resulting stimulation of CD4⁺ T cells [134]. Additionally, at least two complement proteins, C3 and C1q, have been documented to enhance the cross-presentation of IgG-complexed antigens although whether Fc γ R are required for this modulation remains unresolved [135, 136]. An additional layer of complexity to be considered is the nature of the antigen contained within the immune complex, particularly if this contains ligands for additional PRR that might be triggered during or subsequent to Fc γ R-mediated internalization. Specifically, the TLR9 activating ligand CpG has been found to stimulate DC cross-presentation of IgG-complexed antigen by Fc γ RI but to inhibit Fc γ RIIa-driven antigen presentation by pDC [137, 138]. While the effect of other TLR ligands specifically on the cross-presentation of IgG-complexed antigen remain unexplored, ligation of many other TLR ligands, including TLR2, TLR7, TLR9, are known to affect cross-presentation and could thus alter how IgG-bound antigen is processed [64, 67, 139–141].

Despite such potentially mitigating variables, it is clear that uptake of IgG-complexed antigens enhances cross-presentation in the majority of cells. The importance of the initial uptake event in determining the eventual fate of an antigen has been clearly documented, particularly as it pertains to receptor-mediated endocytosis or phagocytosis by molecules such as Fc γ R [40, 52]. Critically, however, the ability of cells highly enabled for cross-presentation, such as CD8⁺ DC, cannot be explained by differential antigen capture and different DC subsets (CD11c⁺ DC vs. Langerhans cells) which take up identical antigen in different manners are able to cross-present antigen with equivalent efficiency [42, 142].

While Fc γ R are highly efficient at binding IgG at the neutral pH levels found at the cell surface and in driving the subsequent internalization of IgG IC, Fc γ R–IgG binding is increasingly impaired as pH drops into the acidic range typical of endolysosomal compartments [28]. While this may not affect the ability of Fc γ R to contribute to cross-presentation of antigen in CD8⁺ DC, where a near-neutral endosomal pH is maintained, it is unlikely that Fc γ R can contribute to active intracellular routing of IgG IC in most other subsets of DC as well as in monocytes and macrophages. While these latter cell types have not been described as efficient cross-presenters of soluble antigen, they are widely known to be capable of presentation of exogenous antigens on MHC class II molecules in a process that is enhanced by IgG complexing of the ligand [68, 143, 144]. Given that such IC would not be expected to be actively trafficked by Fc γ R within these cells, it is vitally important to consider the contribution of receptor-mediated intracellular IgG trafficking in all cells where antibody has been shown to enhance antigen presentation.

Receptor-mediated intracellular trafficking of IgG IC optimizes cross-presentation

Receptor-mediated intracellular IgG trafficking was first described in epithelial cells, where FcRn was demonstrated to be capable of bidirectionally translocating IgG and IgG IC across a polarized cell with distinct apical and basal membrane surfaces in both in vitro model systems and in vivo in the intestines [107, 145, 146]. This has subsequently been confirmed in other polarized epithelia and is a common property of such cell types when FcRn is expressed [147–154]. Several details regarding the biology of FcRn strongly suggested that it might be an attractive candidate as an intracellular IgG receptor within DC capable of driving Fc γ R-internalized IC towards cross-presentation. Chief among these were its lifelong expression in both human and murine DC, its predominantly intracellular distribution and its ability to bind IgG in the acidic pH range typically found within the endosomal system of APC [47, 155, 156]. Furthermore, FcRn is known to increase the efficiency of MHC class II presentation of IgG-complexed antigens above that for soluble antigen in both DC and macrophages, thereby confirming its ability to intracellularly route IgG IC within APC [9, 157]. A series of both in vitro and in vivo data generated using either FcRn-deficient DC or genetically altered IgG incapable of FcRn binding clearly indicated that FcRn ligation greatly increased cross-presentation efficiency for IgG IC-containing antigen of various sizes [28]. FcRn-driven augmentation of cross-presentation was strongest at very low concentrations of antigen and was strictly dependent on initial IgG IC internalization by Fc γ R based upon studies which utilized mutation N297A as a means to disable this type of IgG binding [28]. In fact, at high antigen doses in the range at which soluble antigen are efficiently cross-presented, FcRn did not contribute to cross-presentation by either DC or macrophages [28, 43, 44, 124, 157, 158]. Critically, FcRn-mediated enhancement was predominantly seen in CD8⁺CD11b⁺ DC, which are not typically known to cross-present soluble antigen but in which the pH of the endolysosomal system has been demonstrated to reach acidic conditions permissive for FcRn–IgG binding but inhibitory for Fc γ R–IgG binding [28]. Indeed, while FcRn–IgG binding remained stable at pH 6.5–5.0, the pH of the phagosomal compartments in which FcRn localized was shown to stabilize at approximately pH 6.0 thereby enabling FcRn to remain bound to IgG IC in these compartments for extended periods of time [28]. This is in stark contrast to the endosomal pH conditions of CD8⁺CD11b⁺ DC which are strictly buffered at approximately pH 7.0 by the actions of the NADPH oxidase NOX2 and do not decrease to levels in which FcRn is capable of IgG binding [43, 47].

Unexpectedly, FcRn ligation of IgG IC was found to participate not only in the acidification of the IC-containing phagosome but also in the regulation of the oxidative environment within this compartment since V-ATPase, Rab27a and gp91phox were all found to be

enriched in the vesicles where FcRn was cross-linked [28]. The mechanism of this enrichment remains unknown and could involve either the active recruitment of these molecules, regulation of the fusion and/or maturation of different endocytic compartments, or the active trafficking of IgG IC into a specialized intracellular compartment. Consistent with all previous reports of Fc γ R-mediated cross-presentation of IgG-complexed antigen, antigenic degradation of FcRn-trafficked IC was shown to involve typical ER proteins, to depend upon export of the antigen from the initial compartment into the cytosol and to require proteasomal processing [58, 88, 159]. The observed prolonged retention of FcRn-bound IgG IC within the intracellular compartment is also consistent with another published study demonstrating that IgG complexed antigens are stored for extended periods of time within intracellular vesicles capable of acting as antigen reservoirs for continuous loading onto MHC class I [160]. While this observation may at first seem at odds with the need for proteasomal processing of the IgG-delivered antigen, a plausible explanation is that FcRn-ligation of IgG IC permits a slower and more controlled antigenic degradation in a manner akin to that which is necessary for epitope preservation and efficient cross-presentation of soluble antigen by CD8⁺ DC [19, 24, 28, 43]. As outlined in Figure 1, the combined effects of efficient Fc γ R-mediated internalization and FcRn-directed trafficking within DC subsets not typically conducive to cross-presentation of soluble antigen thus transform these cells into sensitive and potent primers of cytotoxic CD8⁺ T cell immunity.

Implications of the cross-presentation of IgG IC in health and disease

The implications of IgG's ability to augment the cross-presentation of opsonized antigens and drive the subsequent priming of CD8⁺ T cell responses should not be underestimated. Cross-priming of IgG-complexed self-antigens has been shown to contribute to the pathogenesis of autoimmune diseases such as diabetes, where engagement of the inhibitory Fc γ RIIb by IgG IC contributes to regulating development of both systemic and oral tolerance by a mechanism which at least partly involves suppression of cross-presentation by simultaneously ligated activating Fc γ R [126, 128]. Failure of this regulatory system can lead to loss of tolerance to self-antigens, as evidenced by the fact that administration of anti-OVA IgG to mice whose pancreatic β cells express OVA leads to the development of CD8⁺ T cell driven diabetes [161]. Furthermore, IgG IC have been shown to drive colitis in an FcRn-dependent manner and the recent identification of a potential pathogenic role for CD8⁺ T cells in this disease is likely attributable at least in part to activation of CD8⁺ T cells by cross-presentation of IgG-complexed bacteria or bacterial ligands [28, 115, 162]. Indeed, it is likely that the modest ameliorative effect of intravenous immunoglobulin (IVIg) treatment when administered to patients with IgG-mediated diseases such as inflammatory bowel disease (IBD) functions at least partly through inhibition of the cross-presentation of IgG-complexed autoantigens [115, 163–167]. Likewise, inhibition of SYK signaling downstream of Fc γ R activation in DC has been shown to protect mice against the development of autoimmune diabetes, the onset of which was previously demonstrated to depend upon IgG-mediated presentation of self-antigen [161, 168]. Conversely, IgG-mediated cross-presentation is protective towards *Salmonella* infections and can be presumed to contribute to the defense against other pathogenic diseases and viruses [69, 169]. Together, these studies highlight the fact that the cross-presentation of IgG IC occurs under physiological contexts for both IgG bound soluble antigens, such as proteinaceous self-antigens [126, 128, 161], as well as IgG bound particulate antigens, such as microbes [28, 69, 115, 162, 169]. Further, they emphasize the importance of IgG IC-induced cross-priming in both homeostatic and inflammatory immunity. Indeed, it is likely that Fc-receptor-mediated cross-presentation is a central component of immune responses against a wide range of pathogens, particularly intracellular microbes, and aberrant self-cells, such as cancers. While details of the specific contribution of the individual Fc receptors to this process remain to be fully explored, it can be expected that perturbations in either IgG IC

uptake or intracellular routing would disrupt cross-presentation and impair the host's ability to respond in these circumstances.

Perhaps the aspect of IgG IC-driven cross-presentation which has generated the most attention is its therapeutic applications—namely the potential to use low levels of IgG complexed antigen to induce potent cytotoxic CD8⁺ T cell responses against bacterial, viral and tumor antigens. At least two separate studies have demonstrated the importance of anti-viral IgG in driving cross-presentation-mediated protection against immunodeficiency viruses. In the first of these, anti-p55-Gag IgG enhanced the presentation of SIV Gag on MHC class I and improved the generation of specific cytotoxic T cell precursors in a process requiring proteasomal degradation [170]. In the second study, sublingual vaccination with HIV-1 proteins and an adjuvant led to the development of both antigen-specific IgG and CD8⁺ T cells, the latter of which accumulate in the vaginal mucosa [171]. Furthermore, numerous studies have demonstrated the successful generation of anti-tumor CD8⁺ T cell immunity using vaccines composed of either whole, inactivated or apoptotic tumor cells opsonized with IgG and thereby efficiently targeting tumor antigens for presentation on MHC class I molecules without the limitation of needing to first define individual tumor antigens [172–175]. While this technique is not without limitations, it highlights the potential benefits which can be gained from a better understanding of the cross-presentation of IgG-complexed antigens and which will allow for more effective therapeutic manipulations of this potent system.

Cross-presentation by other immunoglobulin receptors

Given the potency of IgG IC-mediated cross-presentation and the prevalence of receptors for other immunoglobulin isotypes, determining whether these other Ig are also capable of driving antigen towards a cross-presented fate will be important to explore. For some isotypes, data already exists on their ability to stimulate CD4⁺ T cells and thus a precedent has been set for their ability to activate cell-mediated immunity. Specifically, IC formed with IgA and antigen have been shown to promote antigen loading onto MHC class II molecules in an Fc α RI (CD89)-dependent manner which was enhanced by signaling from the common γ -chain [176, 177]. Similarly, IgE is known to be able to drive antigen internalization in a manner which drives the activation of CD4⁺ T cells [178–180]. Recently, an insightful review has presented the as yet unproven idea that cross-presentation of antigen bound to IgE and internalized via either or both Fc ϵ RI or Fc ϵ R2 is the mechanism by which IgE-driven responses can be linked to CD8⁺ T cell activation [181]. Furthermore, targeting antigen for receptor-mediated uptake on B cells via surface-expressed IgM can introduce the antigen into a cross-presentation pathway in B cells [182]. Perhaps most importantly of all is the consideration that immune complexes are likely to simultaneously contain multiple different Ig isotypes such that multiple Fc receptors would be triggered upon IC binding and uptake. Indeed, at least one study has demonstrated that both Fc ϵ RI and Fc γ R contribute to the development of atopic dermatitis, although each receptor was found to drive a different aspect of the pathology [183]. Thus, in addition to investigating the ability of different Ig and their receptors to singularly drive cross-presentation, a true understanding of the impact of Ig in both physiological and pathophysiological situations will require an investigation of the cooperative effects of the different Ig–receptor pairs when simultaneously ligated within a cell.

Conclusions

The complexity of the vertebrate immune system is such that coordination between its different branches is required in order to optimize protection against potential pathogens. Due to their dual nature which combines the ability to specifically bind unique ligands with

a conserved Fc region, immunoglobulins are capable of bridging a gap between innate and adaptive immunity. This is particularly the case for IgG, an abundant Ig for which various different receptors exist over a distribution of subcellular localizations. When the Fc region of IgG within an immune complex is bound by their specific receptors on DC, the antigen is delivered into an intracellular pathway allowing for efficient processing and subsequent presentation of epitopes specific for the unique antigen. Thus, an antigen-specific T cell response can be generated by a cell entirely lacking a specific receptor for the antigen. A final aspect that deserves mention is that since IgG-containing immune complexes can potentially deliver antigen to both MHC class I and MHC class II antigen presentation pathways in DC, the DC which contains both internalization (Fc γ R) and routing (FcRn) receptors is able to assume the critically central role of a single site for CD4⁺ and CD8⁺ T cell docking that allows for optimal priming of both helper and cytotoxic responses; the latter of which depends upon T cell help from CD4⁺ T cells (Fig. 2) [20–22]. While we have only begun to understand the mechanisms behind IgG-mediated cross-presentation enhancement and how it compares to that of soluble antigen, the exquisite sensitivity demonstrated for this system makes it an attractive target for further study. Not only will this generate a better understanding of how this process naturally confers immunological protection under conditions of homeostasis or infection but also of how it can be therapeutically manipulated to target and resolve a multitude of pathological conditions.

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Fig. 1.

IgG immune complexes are directed into a cross-presentation processing pathway via sequential interaction with IgG receptors. IgG-containing immune complexes (IgG IC) are bound by Fc γ R on the surface of DC, leading to their initial internalization. In the acidic environment of the endosomal system, generated at least in part by the actions of V-ATPase, the IgG IC is released from Fc γ R but tightly bound by FcRn, which only binds to IgG at pH 6.0. By an as yet unknown FcRn-dependent mechanism, the IgG IC is shuttled into a vesicular compartment equipped with oxidative machinery (gp91phox), delivered in a Rab27a-dependent manner, antigen exporting molecules (Sec61), peptide importing molecules (TAP1/2) and MHC class I itself. Subsequent to cytoplasmic proteasomal processing, epitopes from the antigen initially imported by the IgG IC are loaded onto MHC class I and trafficked to the cell membrane where they may engage CD8⁺ T cells, thereby priming cytotoxic T cell-mediated immunity

Fig. 2.

Delivery of antigen within an IgG IC leads to effective priming of both CD4⁺ and CD8⁺ T cells by the same DC. FcγR and FcRn cooperate to deliver IgG IC into antigen processing pathways conducive to the generation of epitopes for loading onto both MHC class I and MHC class II. This enables activation of CD4⁺ T cells, which, in turn, provide the additional stimuli required for efficient activation of CD8⁺ cytotoxic T cell responses

Table 1

Contribution of known IgG receptors to cross-presentation of IgG IC

IgG receptor	Cell localization	Effect on cross-presentation	Cell type	References
Fc γ R	Plasma membrane	+		[85, 86, 122, 133, 134]
m/hFc γ RI	Plasma membrane	+	CD8 ⁺ DC, CD8 ⁻ CD11b ⁺ DC, pDC	[119, 129, 136]
m/hFc γ RIIa	Plasma membrane	+	CD8 ⁺ DC, CD8 ⁻ CD11b ⁺ DC, pDC	[119, 123, 129]
mFc γ RIIb	Plasma membrane	–	CD8 ⁺ DC, CD8 ⁻ CD11b ⁺ DC, pDC	[124, 126]
m/hFc γ RIII	Plasma membrane	+	CD8 ⁺ DC and CD8 ⁻ CD11b ⁺ DC	[119, 127, 129]
mFc γ RIV	Plasma membrane	?	?	
mFcRn	Endolysosomal vesicles	+	CD8 ⁻ CD11b ⁺ DC	[26]