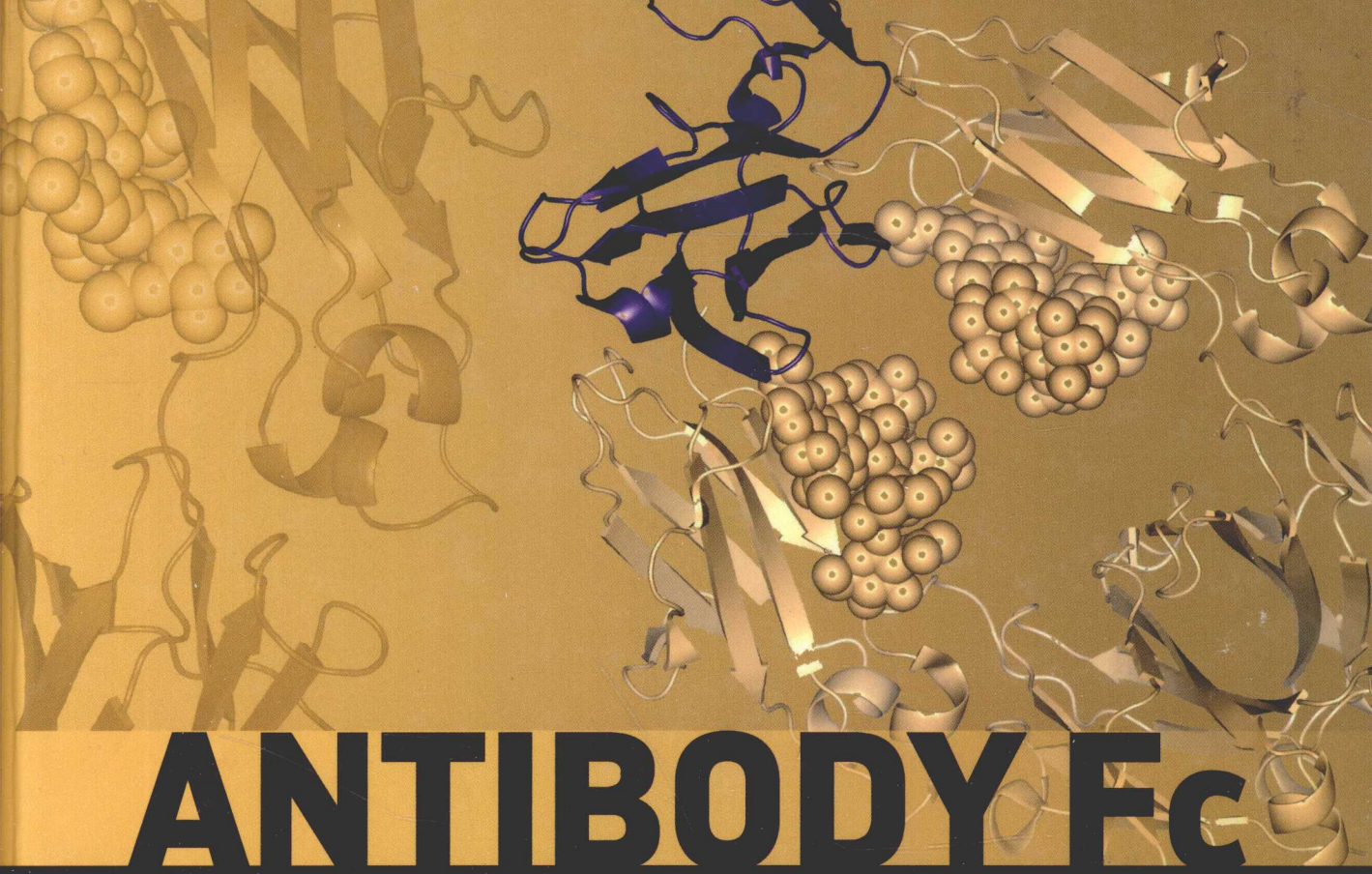

ANTIBODY Fc

LINKING ADAPTIVE AND INNATE IMMUNITY

MARGARET E. ACKERMAN
FALK NIMMERJAHN

ANTIBODY Fc: LINKING ADAPTIVE AND INNATE IMMUNITY

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CHAPTER

1

Antibody-Dependent Cellular Cytotoxicity (ADCC)

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BRIEF HISTORY OF ADCC

In the 1960s, several independent laboratory observations indicated that cells could be killed by other cells, yet the mechanisms of killing were unknown. Several hypotheses were formulated and experiments were conducted paving the way for the discovery and characterization of what we now know as cytotoxic T lymphocytes (CTLs) and natural killer (NK) cells. However, early experiments pointed to the hypothesis that immune serum was in some cases necessary for some types of effector cells to mediate killing of target cells. In 1965, Erna Möeller, a researcher working at the Karolinska Institute, showed that incubation of mouse tumor target cells with heat-inactivated anti-serum from rabbits immunized with these tumor cells, followed by incubation with lymphoid cells from unimmunized mice, resulted in cytotoxicity.[1] Such cytotoxicity required contact or "serum-induced aggregation" between the tumor targets and the lymphoid effectors. Experiments were subsequently performed to identify the aggregating and cytotoxicity-inducing factor contained in serum. In 1970, MacLennan, Loewi, and Harding, researchers working at the Canadian Red Cross Memorial Hospital, reported that the serum factor required for this type of cell-mediated cytotoxicity was an immunoglobulin with the "chemical properties of IgG."[2] Subsequent experiments confirmed this finding by showing that the antibody required belonged to the IgG class and that the mechanism of antibody-dependent killing of target cells by serum factors did not require the heat-labile components of complement, as heat inactivation of serum maintained the killing effect.[2] The phenomenon acquired the name of "antibody-dependent lymphocyte-mediated cytotoxicity"[3] and gradually became known as "antibody-dependent cell-mediated cytotoxicity," or ADCC. The three basic components of ADCC were recognized as being effector cells, antibodies, and target cells coated with antigen. "Targets" could refer to

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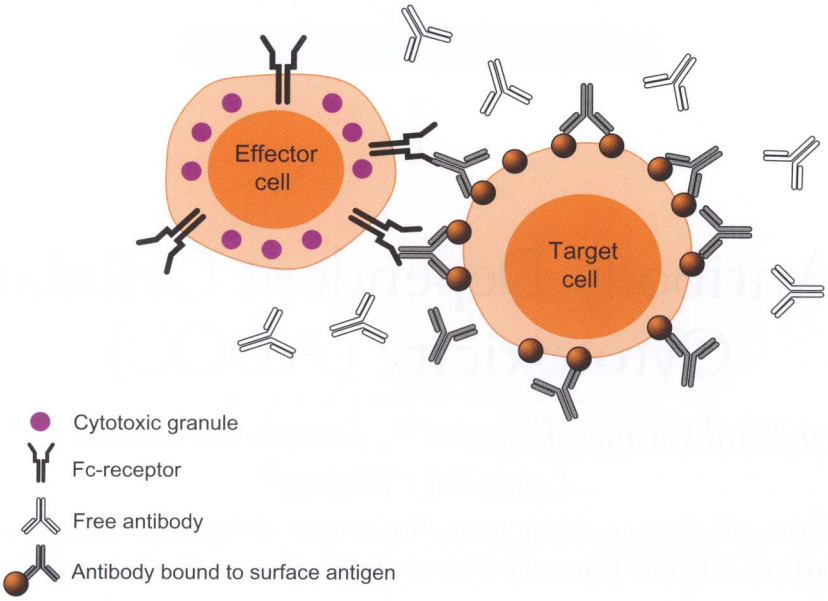

FIGURE 1.1 **Effector cells and targets.** ADCC involves the interplay between a granular effector cell and a target cell expressing antigens on its surface. The granular effector cell must express Fc receptors on its surface for ADCC to occur.

cells expressing tumor, viral, or bacterial antigens; therefore, ADCC became known as an immune mechanism that could be potentially protective against certain types of cancers as well as infectious diseases. Considerable progress has been made in characterizing the effector cells and the receptors involved in this phenomenon, and ADCC can now be defined as the immune mechanism through which Fc-receptor-bearing effector cells can kill target cells that have surface antigens complexed with antibody (Figure 1.1).

EFFECTOR CELLS

Although initial experiments focused on large granular lymphocytes as the main effector cells mediating ADCC, several groups have now characterized the types of cells that can mediate ADCC effector function. Table 1.1 describes the various types of effector cells that have been shown to mediate ADCC. Three common characteristics of these cells are that they are all leukocytes, they contain granules, and they express Fc receptors. Mononuclear leukocytes (NK cells, macrophages, γδ T cells) and polymorphonuclear leukocytes (neutrophils, basophils, eosinophils) can both mediate ADCC.[4] This diversity of effectors is worth emphasizing, as a significant proportion of ADCC experiments reported in the scientific literature are either "NK-centric" or focused on the use of the peripheral blood mononuclear cell (PBMC) fraction to obtain effector cells. This unintentional experimental bias tends to overlook the role of neutrophils and other polymorphonuclear leukocytes (PMNs or PMLs); it is probably a consequence of both the relative ease of working with peripheral NK cells and the practical difficulties associated with working with PMNs, which are rather short lived

TABLE 1.1 Peripheral Blood Effector Cells and Fc Receptors Involved in ADCC

Leukocyte Fraction	Effectors	Predominant Fc Receptors	Refs.
PBMC	NK cells	FcγRIIIA/CD16	Wallace et al.[4]
	Monocytes/macrophages	FcγRI/CD64 FcγRII/CD32	Wallace et al.,[4] Tudor and Bomsel[5]
	γδ T cells (subset)	FcγRIIIA/CD16	Chen and Freedman[120]
PMN	Granulocytes (neutrophils, basophils, eosinophils)	FcγRII/CD32 FcγRIIIb/CD16b FcαRI/CD89	Wallace et al.,[4] Horner et al.[6]

and may require isolation through cumbersome Percoll-gradient procedures or hypotonic lysis steps. While the effector cell phenotype of NK cells, phagocytes, and B are discussed in detail elsewhere in this book, cells contained in the PMN fraction may be equally important effectors involved in Fc-mediated functions, including ADCC.

RECEPTORS INVOLVED

Three types of Fc receptors are involved in mediating IgG-dependent ADCC: FcγRI (CD64), FCγRII (CD32), and FcγRIIIA (CD16). Of these, FcγRIIIA (CD16) is often invoked as the main receptor involved, as it is expressed predominantly by NK cells (Table 1.1); however, *in vitro* evidence indicates that monocytes and granulocytes can mediate equally potent ADCC via other Fc receptors.[4–6] In cancer and infectious disease research, all three FcγRs have been shown to mediate ADCC. Natural polymorphisms in the Fc receptors have been shown to have a clear impact on ADCC *in vitro* and an effect on ADCC-dependent cancer immunotherapy. Additionally, IgA-dependent ADCC has also been described in some models and is dependent on the Fc alpha receptor (FcαR, CD89), which is expressed primarily on PMN and monocytes (Table 1.1).

Our knowledge of Fc-receptor expression and ADCC function to date has been limited to the study of either immortalized effector cell lines or fresh effector cells circulating in peripheral blood. Less is known about Fc-receptor expression in cells residing in mucosal tissues, which represent the first line of defense against invading pathogens. For example, a recent study examining Fc-receptor expression in a limited number of patients (n=5) showed that CD16, CD32, and CD64 expression was virtually nonexistent on rectal macrophages compared to the levels of expression observed on peripheral blood monocytes.[7] Vaginal macrophages from the same patients, however, expressed very high levels of CD16. This may have important implications for ADCC, as it could suggest that ADCC (and other Fc-receptor-dependent mechanisms of immunity) may be relevant as a first line of mucosal defense in some compartments but not in others. In this regard, several studies have examined the role of mucosal antibody in mediating ADCC *in vitro* using effector cell lines or fresh effector cells derived from peripheral blood. In contrast, less is known about the ADCC function of effector cells recovered from mucosal tissues, and defining the expression of Fc receptors across mucosal tissues and assessing their *ex vivo* ADCC function might yield insights into the spatial and temporal role of ADCC in infection and immunity.

MECHANISMS OF ADCC

Recognition of the Target Cell and Cross-Linking of the Fc Receptor on the Effector Cell

An obvious prerequisite for ADCC to occur is the interaction of antibody bound to the target cell with Fc receptors on the effector cell. This interaction is both regulated and facilitated by conformational changes that occur in the antibody molecule only after it has bound to its cognate antigen (Figure 1.1). After binding to surface antigens on the target cell, conformational changes occur in the Fc region of the antibody, which result in its increased affinity for a single Fc receptor on the effector cell.[8] Glycosylation of the Fc region also plays an important role in modulating the affinity of antibody for Fc receptors; in particular, antibodies that are heavily fucosylated (during posttranslational modifications within the B cell) have decreased affinity for FcγRIIIA (CD16), whereas removal of fucose enhances their affinity for FcγRIIIA and their ability to mediate ADCC.[9] Recent evidence indicates that binding between IgG and FcγRIIIA involves tight carbohydrate–carbohydrate interactions that are weakened or obliterated when IgG is fucosylated.[10]

Compared to small, soluble antigens, the relatively larger size of tumors or virally infected cells coated with several antibody molecules on their surface can facilitate physical rearrangements and interactions between Fc receptors present on effector cells (Figure 1.2A). These interactions are often referred to as Fc-receptor ligation, agglutination, aggregation, or cross-linking. The main model to study "ADCC-like" signal transduction pathways relies on the assumption that the first step in generating an ADCC response is the ligation or cross-linking of Fc receptors on the surface of the effector cell as facilitated by a large, "particulate" antigen such as a viral-infected cell coated with surface antigen-specific antibody (Figure 1.2A). Experimentally, to simulate particulate antigen-induced Fc-receptor cross-linking, many researchers incubate NK cells with FcγRIIIA-specific antibodies, followed by incubation with a secondary antibody (Figure 1.2B).[11] Another method of simulating antigen-induced Fc-receptor cross-linking is by "reverse ADCC," an experimental setup in which the polarity of the bridging antibody is reversed (Figure 1.2C).[12] Using these two CD16-cross-linking simulation strategies, ADCC-like signal transduction pathways have been dissected in both human and murine NK cells.

Downstream Signals in the Effector Cell

For ADCC to occur, molecular signals must also be transduced when an FcγR-bearing effector cell recognizes an antibody-coated target cell.[13–15] Much of what we know about ADCC signal transduction is based on experiments using FcγRIIIA-bearing NK cells as effector cells. Less is known about signaling in other effector cells expressing other FcγRs.

In the current signaling model, the gamma (γ) subunit associated with the FcγRIIIA receptor plays a crucial role in signaling (Figure 1.3). It contains immunoreceptor tyrosine-based activation motifs (ITAMs), which are consensus sequences containing tyrosine residues that can be phosphorylated. ITAMs do not have intrinsic tyrosine kinase activity; instead, they become phosphorylated by cellular src kinases upon FcγRIIIA cross-linking. Phosphorylated ITAMs recruit the spleen tyrosine kinase (Syk) protein, which binds to the ITAMs via its SH2 domains and becomes activated (Figure 1.3). Recruitment and activation of Syk triggers three main pathways involved in ADCC: phospholipase C-gamma pathway (PLC-γ), phosphatidylinositol 3-kinase (PI-3K) pathway, and Vav/Rho-family G-proteins pathway.

The PLC-γ pathway involves the Syk-dependent phosphorylation of the PLC-γ isozymes. Activated PLC-γ cleaves membrane

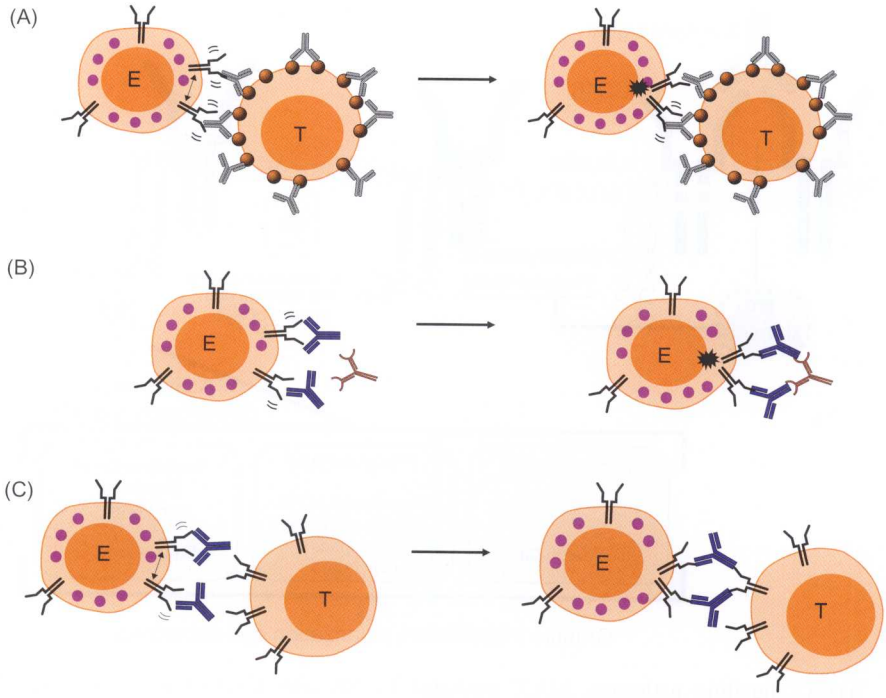

FIGURE 1.2 **Antibody and FcgR ligation.** ADCC involves cross-linking Fc receptors on the surface of effector cells. (A) The particulate nature of antibody complexed to antigens expressed on the surface of a target cell facilitates the physical rearrangement of Fc receptors, bringing them closer to each other and inducing their cross-linking (represented here by a black burst symbol). (B) ADCC in human NK cells can be simulated *in vitro* by incubating human NK effector cells with a mouse anti-human FcγRIIIA (mouse anti-human CD16) antibody (blue), followed by incubation with a secondary antibody (red), such as goat anti-mouse IgG. Addition of the secondary antibody will facilitate physical interactions between CD16 molecules, leading to their cross-linking. (C) ADCC in human NK cells can also be simulated *in vitro* via "reverse ADCC"—that is, by first incubating human NK effector cells with a mouse anti-human FcγRIIIA (mouse anti-human CD16) antibody (blue), followed by incubation with a mouse target cell expressing FcγRI receptors (gray). The particulate nature of the target cell will also facilitate physical interactions between CD16 molecules, leading to their cross-linking. Note the reversal in antibody polarity between (A) and (C). *E*, effector cell. *T*, target cell.

phosphatidylinositol 4,5-bisphosphate (PIP_2) to generate inositol-1,4,5-trisphosphate (IP_3) and diacylglycerol (DAG). IP_3 triggers an increase in intracellular free calcium, which is required for cytotoxic granule mobilization and exocytosis. DAG activates protein kinase C (PKC), which is necessary for other NK cell functions not directly related to ADCC. The PLC-γ pathway is required for ADCC function and can be blocked by herbimycin A, an antibiotic that inhibits tyrosine kinase activity and ADCC.

The PI-3K pathway involves the Syk-dependent activation of PI-3K. PI-3K is a heterodimer composed of an 85-kDa adaptor protein (facilitates interactions with other tyrosine kinases) and a 110-kDa catalytic subunit. PI-3K phosphorylates membrane PIP_2 into membrane phosphatidylinositol 3,4,5-triphosphate (PIP_3). PIP_3 mediates recruitment of pleckstrin homology (PH) domain-containing signaling proteins—that is, proteins (such as PLC-γ) that can be recruited to the membrane through binding

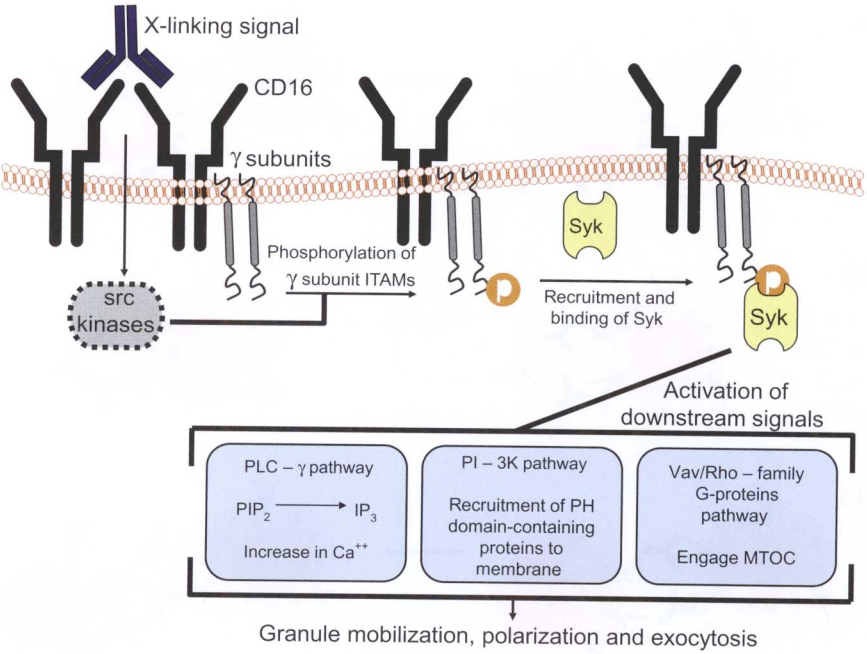

FIGURE 1.3 **ADCC signaling pathways.** ADCC mediated by NK cells involves three main signaling pathways. Following CD16 cross-linking, src kinases are activated and phosphorylate the ITAMs on the γ subunits associated with CD16. This is followed by recruitment of Syk and subsequent activation of the PLC-γ pathway, the PI-3K pathway and the Vav/Rho-family G-proteins pathway. The concerted action of these pathways leads to granule mobilization and exocytosis toward the target cell.

to membrane-bound PIP_3. Recruitment of PH domain-containing proteins to the membrane facilitates their interactions, thereby physically enhancing signal transduction pathways. The PI-3K pathway is also required for ADCC function and can be blocked by wortmannin, a fungal metabolite that inhibits ADCC and binds covalently to the p110 subunit of PI-3K.

The PH domain-containing Vav protein and the Rho family guanine nucleotide-binding proteins (G proteins) are also involved in NK cell granule polarization required for ADCC function. Syk is involved in the phosphorylation of Vav, although the precise mechanism remains unclear.[13,15] The cholera toxin A subunit (Ctx-A), a known inhibitor of G proteins, can inhibit ADCC activity; however, it is unclear where exactly this inhibition occurs, as Ctx-A may also have pleiotropic effects on IP_3 and GTP-binding proteins.[16]

For the sake of simplicity, it is tempting to limit the description of ADCC signaling mechanisms to the three Syk-related pathways outlined in Figure 1.3; however, we must bear in mind four important concepts to avoid oversimplifications or dogmatic views of the downstream signals involved in ADCC:

1. Signaling cascades do not work in isolation, and there is a considerable degree of cross-talk between Fc receptors and integrins,[17] surface receptors, and other intracellular proteins whose functions are still under investigation.[18]
2. There is a considerable degree of redundancy involving certain steps along these three pathways.
3. ADCC signaling mechanisms involving FcγRI, FcγRII, and FcγRIIIb have yet to be