Abstract: The evolution of the immune system has provided a multilevel system that interconnects the innate and adaptive immune systems to serve at least three central purposes: the defense from microbial pathogens, the capacity for discrimination of self- from non-self necessary for the prevention of autoimmune disease, and essential effector roles in wound repair and tissue remodeling. In recent studies, we have elucidated an unsuspected role for a class of naturally occurring autoreactive antibodies from the most primitive tier of B lymphocytes, which regulates fundamental functions of the innate immune system. Our findings also throw light onto long unresolved mysteries regarding the origins of the earliest waves of B lymphocyte development. [Discovery Medicine 8(42):151-156, October 2009]

How and Why Are We Born with Antibodies?

Antibodies produced by B lymphocytes provide key functions in the immune system to help in the protection against pathogens by their capability for recognizing infectious agents, toxins, and other factors. At birth, we already have substantial levels of circulating IgM antibodies that are poised and ready to contribute to neonatal host defenses from the external world and the many associated threats. Unlike the IgG and IgA antibodies that come from the maternal immune system, these IgM antibodies, denoted natural antibodies (NAb), arise in the sterile (but not antigen-free) womb and are produced by neonatal B lymphocytes. B cells clonally express antibodies both as soluble IgM antibody proteins and as B-cell antigen receptors (BCRs) on the cell surface. In principle, each B cell expresses a specific BCR variant that is composed of heavy and light chains with a unique amino acid composition that creates the specific antigen recognition capability of the clonally distributed receptor. During immune development, the BCR of a B cell is generated by the combinatorial recombination of three types of gene segments for the BCR heavy chain and two types of minigenes for kappa or lambda light chains, with an overlay of a range of effective molecular diversification mechanisms. Taken together, these somatic mechanisms can mold limited panels of inherited genes into an immense number of potential BCR structures (>10^{18}), which far exceed the finite number of lymphocytes in the body (~10^{10}).

With this capacity to generate an effectively limit-
less range of antigen receptors, one might predict that each individual would make receptors for the same antigens that would be structurally unrelated to those made in other individuals. Yet, opposite of what might have been predicted, mice and humans (and presumably other species) are born with restricted BCR/antibody immune repertoires, and these neonatal B-cell repertoires display frequent recurrences of the same preferential gene rearrangements (Perlmutter et al., 1985; Schroeder et al., 1987) and even antibodies with identical amino acid sequences and heavy-light chain pairings (Seidl et al., 1997). Furthermore, these neonatal B-cell responses appear to derive from a distinct set of mature B cells that are responsible for most circulating IgM (Herzenberg, 1989). Yet, the mechanistic basis for this often remarkable level of conservation of somatically generated immune receptors in neonates has remained incompletely understood. Notably, while it has been shown that certain conserved clones from this neonatal B-cell pool can be important contributors to immune defenses from infectious agents (reviewed in Binder and Silverman, 2003), such foreign antigens are not present in utero. Moreover, as similar neonatal antibody expression patterns are seen in germ-free mice, this suggest that commensal flora are not the primary drivers for the initial antigenic-selection of such highly represented B-cell clones. It is therefore logical to postulate that such patterns of conserved early immune development result from an evolutionary pressure for the initial B-lymphocyte clonal selection of a conserved repertoire that provides important (although not necessarily non-redundant) housekeeping functions.

One of the most important regulatory pressures for B-cell and antibody selection is for the immune system to avoid the generation of autoantibodies recognizing self-antigens that would cause the immune system to attack the body and lead to disease. The conserved neonatal B cell repertoire can therefore seem to be a paradox, which might suggest selection by antigenic-ligands in the body, in the light of the great range of BCR and antibodies that could potentially be made. In recent studies, we have considered this paradox in the context of emerging evidence that some natural antibodies recognize cells dying from apoptosis, which is a form of programmed cell death in which macromolecules are degraded or translocated by specific enzymatic cascades. The key in our studies was to understand what ligands on apoptotic cells are most accessible to B cell and antibody interactions. Interestingly, we now believe that certain B cells appear to be selected by BCR interactions with specific modified cell membrane determinants on cells that are undergoing the physiologic process of apoptotic death (i.e., apoptotic cell membrane (ACM) neo-determinants) (Chen et al., 2009a). Anti-ACM antibodies expressed by these B-cells inherently discriminate apoptotic from healthy cells. As outlined below, we have found that these anti-ACM antibodies have regulatory interconnections with the cells of the innate immune system that use limited sets of cellular receptors, such as Toll-like receptors (TLRs) for the recognition of pattern-associated molecular motifs (PAMPs). These TLRs, and other innate receptors transduce signals to ligands that represent “danger signals” from microbial pathogens and are an important line of defense against invading bacteria and virus. But persistent and overexuberant responses to these signals can also cause considerable damage to the host, so careful regulation is required. We found that these anti-ACM NAb can regulate the fundamental functional capacity of the innate immune cells to TLR agonists, as well as enhance the phagocytosis of apoptotic cells.

Removal of Apoptotic Cell Corpses Is a Key Responsibility of the Innate Immune System

Over a century ago, Metchnikoff first recognized the importance of the homeostatic removal of dead cells, which he termed physiologic inflammation (Metchnikoff, 1891). Apoptotic cells are constantly generated during normal cell and tissue turnover, as well as due to exposure to environmental factors such as smoking or ultraviolet light. Hence, the clearance of apoptotic-cell corpses occurs throughout the lifespan of multicellular organisms and is important for normal development during embryogenesis, as well as a consequence of cellular proliferation and differentiation that continues throughout life as we continue to remodel our tissues, and replace senescent and superfluous cells. In fact, every day, more than 10^{11} cells in our bodies die by apoptosis.

The clearance of apoptotic cells by direct engulf-
ment by phagocytic cells has been termed efferocytosis (taken from the Latin effero, meaning to take to the grave or to bury), as it is distinct from phagocytosis that involves IgG-mediated clearance. This specialized multi-step process involves numerous surface ligands (that provide “eat me” signals), bridging molecules, phagocyte receptors, and signaling transducers (reviewed in Cline and Radic, 2004). In fact, of the 14 death genes of the laboratory worm species, Caenorhabditis elegans, at least half encode proteins that are required for engulfment of apoptotic cells (Ellis et al., 1991) and therefore the engulfment of apoptotic cells is likely to represent one of the most primitive and highly conserved functions of the innate immune system.

Although in health these apoptotic cells do not pose an immediate threat to the host, nonetheless there is an absolute need for rapid and efficient apoptotic clearance to avoid the toxic effects that can ensue from exposure to the internal contents of dying cells. Apoptotic cell clearance has also been linked to the control of the resolution of inflammation that can otherwise be highly detrimental to the host. In fact, it has been postulated that impaired efferocytosis is a contributor to chronic inflammation that underpins many of the most common diseases, from atherosclerosis to chronic obstructive pulmonary disease to rheumatoid arthritis (and many others), which are all associated with overexuberant and dysregulated inflammatory responses.

Higher mammalian species express a large number of soluble factors as part of the efferocytosis pathway of the innate immune system. Although these molecules have seemingly overlapping functional roles for the discrimination of healthy from dead and dying cells, each may be differentially expressed by different cell types and anatomic sites and organs, to facilitate these homeostatic roles and generally also contribute to the recognition of foreign pathogens. Amongst these “eat me” signals are the recognition molecules of the complement system, C1q and the structurally analogous multimeric molecule, mannan binding lectin, MBL (Ogden et al., 2001), which share a common evolutionary progenitor (Matsushita et al., 2004). C1q has been shown to be directly deposited from serum onto late apoptotic cells, which enable recognition and engulf-

Natural Antibodies Can Regulate Key Innate Immune Functions

In recent reports (Chen et al., 2009a; 2009b), we have characterized the immune modulatory properties of murine Abs that recognize ACM determinants. We first showed that apoptotic cell immunization in C57BL/6 mice induced responses dominated by antibodies to the simple compounds, phosphorylcholine (PC) and malondialdehyde (MDA), that modify self-antigens on the surface of apoptotic cells (Chen et al., 2009a). In explanation, PC is a phospholipid head group that is an immunodominant epitope on microbial pathogens, such as S. pneumoniae and others, but the PC head group is also a component of neutral phospholipids (e.g., phosphatidyl choline, PtC) in the outer leaflet of cell membranes. In healthy cells, PC is sequestered and not available for immune recognition. Yet, during apoptotic death the PC head group becomes exposed due to oxidative damage to the polyunsaturated fatty acid side chains of phospholipids, and subsequently generates reactive aldehydes such as POVPC (reviewed in Binder and Silverman, 2005). Thereby, damaged cells are flagged via Ab recognition of PC neo-epitopes. A second major set of IgM anti-ACM Abs recognizes MDA modified epitopes. MDA is a reactive aldehyde that is a major degradation product of interactions of unsaturated lipids with reactive oxidation species. We and others have shown that Ab recognition of ACs via the oxidation-associated phospholipid determinants, PC and MDA, enable the discrimination between cells undergoing apoptotic death from healthy cells (Shaw et al., 2000; 2003; Binder et al., 2003; Chang et al., 1999; Chen et al., 2009a; 2009b).

Much of this anti-PC antibody response in the
mouse appears to come from a well-characterized B-cell clone, the prototypic T15 B-1 cell clonotype, which was defined by H-L paired canonical antibody gene rearrangements without hypermutation. Since the first report appeared over 40 years ago (Cohn et al., 1969), due to the conserved and high-level expression in the murine immune system, this exact B cell clone has been isolated and independently characterized many times (e.g., S107, HPCM2, EO6, and others). T15 B cells reside within the self-replenishing pool of B-1 cells that are the earliest to appear during immune development. In fact, T15 clonotypic B cells has been shown to spontaneously arise and become highly represented within the first week of life, even in mice raised under germ-free conditions, which suggests that microbial ligands are not primary mediators of clonal selection.

While it has long been known that T15-NAbs bind to phosphorylcholine (PC) determinants, and contribute to host defense to PC-containing pneumococci and other microbes, and provide optimal protection from systemic infection. Our studies suggested that apoptotic cells select and expand the T15 anti-ACM antibody in adult mice. We therefore postulate that apoptotic cell determinants that arise during immune development are likely to also contribute to the initial selection and expansion of these lymphocytes during early development.

We also found that this prototypic anti-ACM antibody can affect key steps involved in efferocytosis. Both C1q and MBL were previously shown to be directly deposited from the sera onto apoptotic cells at low levels, but this direct association is relatively inefficient and primarily occurs on late stage apoptotic and secondary necrotic cells. We found that after binding to apoptotic cells, T15 IgM greatly enhanced the recruitment and deposition of C1q. In addition, through interactions with the sugar molecules on this anti-ACM IgM antibody, binding of T15 to apoptotic cells also greatly enhanced the deposition of MBL. This was especially important, because although MBL is well known for its contributions to the clearance of microbial pathogens and apoptotic cells, its potential role in IgM-antibody effector functions has been little explored. Notably, early stage apoptotic cells are known to have the greatest immuno-modulatory properties (Chen et al., 2009a; 2009b).

A main focus of our studies has been understanding how these pathways may affect the immune-regulation of dendritic cells and macrophages. Dendritic cells are especially important as they serve as sentinels within the immune system, and when triggered by recognition of pathogen-associated molecular motifs, these cells rapidly respond by differentiating and secreting a broad range of inflammatory and also chemoattractant factors that draw other immune cells to the site of the response. In addition, like macrophages, dendritic cells can become potent antigen-presenting cells for the recruitment and expansion of antigen-specific T lymphocyte responses. In chronic conditions, these innate cells may be continually drawn into sites of tissue injury and infection, where they continue to stoke the inflammatory flames and they facilitate active immune responses. We found that interactions with anti-ACM antibodies resulted in greatly enhanced phagocytic clearance of apoptotic cells by both macrophages and dendritic cells (Chen et al., 2009b). This capacity was in part based on the capacity to recruit C1q or MBL.

We also wondered how these antibodies might affect inflammatory responses, and found that the monoclonal anti-PC/ACM, T15 IgM, also provided a dose-dependent suppression of responses to stimulation of a wide range of TLR molecules (TLR3, TLR4, TLR7, and TLR9), which recognize ligands as diverse as bacterial endotoxin, naked DNA, and the double stranded RNA of viruses. Notably, this antibody could also suppress dendritic cell production of cytokines and chemokines implicated in the pathogenesis of rheumatoid arthritis and many other chronic diseases. Importantly, we found that infusions in vivo of anti-ACM IgM also suppressed collagen-induced arthritis (CIA) and arthritis induced by autoantibody immune complexes to collagen (Chen et al., 2009b), which are two murine models of inflammatory arthritis that have features of rheumatoid arthritis. From these initial observations, we are currently studying how anti-ACM antibodies may also block inflammatory responses to immune complexes made by pathologic IgG autoantibodies.
Conclusions

Our studies have found unexpected regulatory roles for a class of naturally occurring antibodies that are present from birth. While less understood in humans, in mice these antibodies are reported to come from B-1 cells, the primordial tier of the B-lymphocyte compartment that is the major source of the “non-immune” IgM NAbS constitutively produced throughout life. This distinct set of self-replenishing mature B lymphocytes have been described as innate-like as they express a restricted and recurrent antibody repertoire that may arise by a programmed sequence during immune development (Perlmutter et al., 1985; Schroeder et al., 1987; Herzenberg, 1989). These antibody responses are also very prominent in humans from birth. We have shown that innate-like antibodies to apoptotic cells may have regulatory roles through effects on innate immune cells even at remote sites in the body, and our recent studies suggest they may also act to dampen the pathologic progression of diverse inflammatory diseases. The characterization of the structural features of these antibodies and the molecular requirements for their full activities for such potent anti-inflammatory housekeeping functions is helping us to develop blueprints for a new therapeutic approach, which should be relevant to the treatment of a broad range of conditions that plague the world and the western societies in particular.

References


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