HHS Public Access

Author manuscript

Immunol Rev. Author manuscript; available in PMC 2017 January 01.

Published in final edited form as:

Immunol Rev. 2016 January ; 269(1): 26–43. doi:10.1111/imr.12382.

Apoptotic cell responses in the splenic marginal zone: A paradigm for immunologic reactions to apoptotic antigens with implications for autoimmunity

Tracy L. McGaha[1,2,4] and **Mikael C.I. Karlsson**[3,4]

[1]Department of Immunology, Faculty of Medicine, University of Toronto, Toronto ON, Canada, M5S 1A8

[2]Princess Margaret Cancer Center, University Health Network, Toronto ON, Canada, M5S 1A8

[3]Department of Microbiology, Tumor and Cell Biology, Karolinska Institute, SE-171 77, Stockholm, Sweden

Summary

Apoptotic cells drive innate regulatory responses that result in tolerogenic immunity. This is a critical aspect of cell physiology as apoptotic cells expose potentially dangerous nuclear antigens on the surface in apoptotic blebs, and failure in their recognition, phagocytosis, or destruction can cause dramatic autoimmunity in experimental models and is linked to development and progression of systemic pathology in man. The marginal zone is a specialized splenic environment that serves as a transitional site from circulation to peripheral lymphoid structures. The marginal zone serves a key role in trapping of particulates and initiation of innate responses against systemic microbial pathogens. However in recent years it has become clear the marginal zone is also important for initiation of immune tolerance to apoptotic cells, driving a coordinated response involving multiple phagocyte and lymphocyte subsets. Recent reports linking defects in splenic macrophage function to SLE in a manner analogous to marginal zone macrophages in lupus-prone mice provides an impetus to better understand the mechanistic basis of the apoptotic cell response in the marginal zone and its general applicability to apoptotic cell-driven tolerance at other tissue sites. In this review we discuss immune responses to apoptotic cells in the spleen in general and the marginal zone in particular, the relationship of these responses to autoimmune disease, and comparisons to apoptotic cell immunity in humans.

Keywords

apoptosis; *macrophage*; *tolerance*; *autoimmunity*; *NKT cell*; *marginal zone*; *B cell*; *lupus*

Introduction

The scientific concept of cell death has its origins in the mid 19th century when it was predicted that cells must die during developmental processes, most notably embryogenesis

[4]Address correspondences to: Tracy L. McGaha, Ph.D., tmcgaha@uhnresearch.ca or Mikael C.I. Karlsson, Ph.D., mikael.karlsson@ki.se.

and fetal development (1). Although some of the prominent features of apoptotic cell death including pyknosis (chromatin condensation and marginalization), karyorrhexis (nuclear fragmentation), and membrane budding were recognized early on, it was not until 1971 that the seminal paper by Kerr et al identified a mode of death phenotypically distinct from general necrosis, naming the death process apoptosis the following year (2, 3). However, prior to the formal definition, this type of cell death was already closely linked with inflammation. It was noted at least by the 1960's that large-scale cell death (during embryonic limb development for example) was associated with a massive infiltration of phagocytes (4), thus associating cell death and innate immune responses.

It has been recognized for some time that apoptotic cells could drive classic inflammatory processes; however, this was believed to be an eventual outcome of delayed clearance or other mechanisms promoting persistence of apoptotic cells leading to a “secondary necrotic” reaction (5). Nevertheless, in general apoptotic cells were considered inert particles that were cleared to prevent or, in certain circumstances (e.g. neutrophil clearance), to resolve inflammation (6). Conceptually the understanding of the apoptotic cell/phagocyte relationship changed in the 1990s as it became apparent that efferocytosis (i.e. the process of apoptotic cell phagocytosis) drives an actively suppressive response that antagonizes activating, inflammatory signals (7, 8). At this time, groundbreaking work by Peter Henson's laboratory described a ligand and cognate receptor-dependent immunosuppressive response elicited in macrophages and dendritic cells driving interactions with apoptotic cells and subsequent responses (9–11). This discovery eventually led to the modern definition of immunologically silent cell death that currently includes recognition of apoptosis by a battery of receptors, production of suppressive soluble effector molecules such as IL-10 and TGF-β, acquisition of a tolerogenic phenotype in dendritic cells and macrophages, and durable long-term adaptive immune tolerance to apoptotic cell-associated antigens. The notions of “silent” death and infectious (i.e. transferrable) tolerance are now a dominant feature of research in the field, and attempts to manipulate apoptotic cell-driven suppressive pathways are being attempted in experimental immuno-modulatory therapy for hyper-immune diseases such lupus and multiple sclerosis as well as cancer and other diseases where apoptosis may be a barrier to protective immunity. However, there is a significant deficit in our understanding of the complex cellular and molecular mechanisms underpinning apoptotic cell-driven immune suppression and tolerance *in vivo* and how breakdown of these contribute to autoimmune diseases.

The marginal zone (MZ) of the spleen is a transitional site where the vasculature merges into a venous sinusoidal system. The MZ populated by several innate-like lymphocyte and phagocytic populations that are specialized to monitor the blood, screening for signs of infection such as bacterial polysaccharides and serve a scavenging function to remove particulate material (including apoptotic cells) from circulation. Studies in mouse models lacking apoptotic cell scavenger receptors highly expressed in the MZ (i.e. macrophage receptor with collagenous structure/MARCO or scavenger receptor A1/SR-A) found no defects in either apoptotic cell trapping or immune homeostasis (12). Likewise, mice deficient in the major MZ cellular populations (MZ B cells, $MARCO^+$ and $CD169^+$ macrophages) did not show an impairment of the immune rheostat or development of

spontaneous autoimmunity (13). Thus it was unclear what role responses in the MZ had in apoptotic cell-driven immunity and prevention of autoimmunity either locally or systemically.

Our laboratories have been examining the function of the MZ in apoptotic cell responses for the last 10 years. The studies have revealed key mechanistic roles for MZ-resident cell populations in generation of tolerance after apoptotic cell exposure and prevention of both spontaneous and induced systemic autoimmunity. Moreover, the apoptotic cell response in the MZ has proven to be an incredibly dynamic process that requires the coordinated activity of B cells, NKT cells, macrophages, dendritic cells, and regulatory T cell populations working in parallel and sequentially. This coordinated activity ultimately leads to adaptive immunity including immunoglobulin responses against apoptotic cell antigens and antigen-specific FoxP3^{+} Tregs driving clearance and long-term tolerance.

In this review we focus on immune responses in the MZ as a model of apoptotic cell immunity. While the structure is unique, there are mechanistic similarities with mucosal lymphoid tissue, lymph nodes, and sites elsewhere in the body. Thus, while it is not likely that immunity in the MZ has complete overlap with immune reactions in other tissue locations, there is sufficient commonality to allow application of lessons learned to other sites of efferocytosis and multiple disease models. Moreover, the data derived from this model system has yielded the surprising observation that apoptotic cells are potently recognized by the immune system and it is only active counter-regulatory signals induced in a concomitant fashion that prevent apoptotic cells from driving inflammatory, rather than regulatory, immunity. In this review, we will highlight advances in understanding of the nature of apoptotic cell immunity in the MZ focusing on the novel interactions and links to autoimmune disease.

Apoptosis and tolerance: General themes

Paradigm of silent death

Even in tissues with a high rate of apoptotic turnover such as the thymus and spleen it is difficult to find significant numbers of apoptotic cells. This is due to the magnificently efficient clearance mechanisms driven by professional and non-professional phagocytes. These mechanisms often appear to have overlapping function, as deletion of one or several sensing and/or removal pathways may have minor effects on homeostasis. Nevertheless, genetic deletion approaches have been informative demonstrating that loss of certain critical pathways leads to fulminant inflammation and lethal autoimmunity (14–16).

Studies by Fadok et al. demonstrated that apoptotic cells expose signals that promote phagocytic uptake (9). Later, it was shown that cellular engulfment was a precipitating factor for apoptosis in *Caenorhabditis elegans* (17, 18). In these studies, cells receiving weak apoptotic signals had the capacity to survive unless phagocytosed, suggesting a critical link between efferocytosis and the apoptotic program. Subsequently, Lauber et al. identified the first putative chemotactic signal released by apoptotic cells promoting phagocyte recruitment (19). These concepts led to the hypothesis that apoptotic cell clearance is composed of a discrete, overlapping sequence of events in which apoptotic cells advertise

their status, recruit local phagocytes for rapid clearance, and promote uptake to prevent inflammatory reactions (20).

Chemotactic signals

Currently, identified apoptotic cell-released chemotactic agents fall into three categories: 1) Chemokines- of which the only identified example is CX3CL1/fractlaline (21). 2) Lipids- lysophosphatidylcholine/LPC and sphingosine-1-phosphate/S1P have been identified as potential apoptotic cell-released chemotactic agents (19, 22). However, while LPC release from apoptotic cells can drive phagocyte recruitment, the concentration required does not appear to be physiologic suggesting it is not a bona fide chemotactic agent in situ. 3) Nucleotides- ATP and UTP are released at low levels by apoptotic cells and can serve as chemotactic agents for macrophages (23). Free nucleotides are probably sensed by the purinergic receptor $P2Y_2$, and disruption of $P2Y_2$ function increases apoptotic cell accumulation in the thymus after dexamethasone treatment suggestive of a role in constitutive apoptotic cell clearance (24). However, basal apoptotic cell numbers in the thymus are not affected by $P2Y_2$ inhibition calling the role of nucleotides in homeostatic clearance into question. Moreover $P2Y_2^{-/-}$ mice do not develop autoimmune disease suggesting nucleotide driven recruitment is not a predominant mechanism used for apoptotic cell clearance in vivo.

Studies examining chromatolysis (25) observed that yolk-sac epithelial cells had appeared to phagocytose neighboring cells with apoptotic characteristics (1, 26). Although the significance of this observation was not grasped fully at the time, it is now clear that in many circumstances cells neighboring an apoptotic cells act as non-professional phagocytes driving clearance of cellular debris. In such situations recruitment is likely not required for apoptotic cell uptake. Moreover, many tissues are populated with significant numbers of professional phagocytes suggesting chemotaxis may not be requisite for clearance in many instances. In an alternate view, Ravishandrian has suggested that release of chemotactic agents by apoptotic cells may have an additional/alternative function promoting tolerogenic activation of phagocytes (27). Thus it is possible that recruitment of phagocytes is a relatively context-specific event and the more germane function of chemotactic agents may be to properly activate local phagocytes priming them for efferocytosis.

Apoptotic cell recognition

Recognition of a cell committed to apoptosis is the most critical factor in efferocytosis. This concept has been borne out by numerous findings demonstrating that disruption of receptors or ligands involved in apoptotic cell capture by phagocytes can lead to severe autoimmune and autoinflammatory disease. Recognition is primarily based on four factors. I) Alteration in the lipid topography of the plasma membrane. II) Oxidation of lipids exposed on the surface of the cell and sugar modification (i.e. altered self) (28). III) Surface reduction of so called “don't eat me signals” including complement inactivation pathways. IV) Opsonization by serum proteins such as IgM, complement, and thrombospondin.

Lipid localization on the plasma membrane is highly asymmetrical with chlorine-containing lipids on the outer leaflet (e.g. phosphatidylcholine/PC), and amino-phospholipids including

phosphatidylserine (PS) predominately found on the inner leaflet (29). Early during apoptosis this distribution is altered and PS is rapidly externalized, creating what is arguably the most pathognomonic signal of commitment to the apoptotic process (9). Increased PS exposure occurs within minutes on the surface of apoptotic cells reaching a zenith of a >200-fold increase within 2 hours (30). PS exposure is required for efferocytosis (9); however, activated viable cells can also increase external PS exposure, albeit by mechanisms distinct from apoptotic cells (31–36). Moreover, when viable cells are forced to constitutively present external PS, they are not targets of phagocytosis unless driven to apoptosis (37), suggesting that PS alone is insufficient to signal the presence of an apoptotic cell. Several lipids are targets of peroxidation in apoptotic cells, including the acyl tail of PS (38–40). CD36 is a scavenger receptor involved in apoptotic cell-driven immune suppressive activity that binds oxidized PS (41). Likewise, other scavenger receptors implicated in apoptotic cell phagocytosis (including SRA, CD14, CD68, and MARCO) have an affinity for oxidized lipids promoting capture and delivery of regulatory signals to the phagocyte (41–44). In addition to scavenger receptors, the PS-bridging molecule MFG-E8 can recognize both oxidized and unmodified PS with relatively equal affinity while GAS6 appears to have preferential affinity for oxidized PS (40, 45). It is important to note that other lipid species in the outer leaflet (oxidized and unmodified) have the potential to alter interactions with PS via competitive binding and other mechanisms; thus it is likely the role of PS in apoptotic cell recognition is the net effect of a nuanced interaction of PS and other lipids on apoptotic cells and cognate receptors on the phagocyte.

It has been suggested that PS recognition is the signal for immediate clearance while serum opsonins (i.e. complement, immunoglobulin, pentraxins) are involved in clearance of late apoptotic cells that escape initial PS-dependent capture events (46). Certainly blocking PS recognition delays clearance and partially abrogates the anti-inflammatory impact apoptotic cells have on phagocytes. However, complement rapidly opsonizes apoptotic cells due to reduced surface expression of complement regulators including CD46 (47). CD46, a “don't eat me” signal, inhibits phagocytosis of viable cells by preventing spontaneous C3 activation and complement deposition on the cell surface. When cells undergo apoptosis, CD46 is lost by caspase-dependent release from the plasma membrane (47). Without CD46-driven regulation, C1q and C3b are rapidly deposited on apoptotic cells although this does not lead to assembly of the downstream membrane attack complex. C1q has long been known to bind DNA (48), and DNA is rapidly exposed on the surface of apoptotic cells localizing to blebs presumably promoting recognition and uptake (49–51). Elward et al. found treating apoptotic cells with nuclease was sufficient to abrogate complement deposition (47), suggesting that although C1q has been reported to bind with PS-containing complexes (52), C1q-binding to nucleic acids is the more germane interaction for apoptotic cell efferocytosis.

Several scavenger receptors promote apoptotic cell capture by interaction with C1q in marginal zone phagocyte populations including SIGN-R1, a c-type lectin highly expressed by MZ macrophages (Mφs) (53), and SCARF1, a class F scavenger receptor expressed by $CD8\alpha^+$ MZ DCs (54, 55). In the case of SCARF1, genetic deletion resulted in elevated apoptotic cell burden and development of lupus-like disease in mice suggesting a critical role in apoptotic cell removal and tolerance in the periphery (55). Homozygous deficiency of C1q is a rare genetic lesion, however this defect has one of the strongest linkages to

autoimmunity known, with a >90% risk of developing severe lupus-associated pathology (56, 57). Taking all of this into account, it must be assumed that while PS exposure is certainly a key feature of efferocytosis, recognition and clearance mechanisms will be diverse reflecting the context of cell death including the type and activation state of the dying cell as well as location. Moreover, it implies in circulation opsonins targeting a variety of apoptotic cell exposed targets, rather than direct PS recognition, may play a dominant mechanistic role in removal and prevention of autoimmunity.

TLRs and nucleic acid sensing in apoptotic cell responses

The biologic importance of PS recognition in apoptotic cell immunity is clearly demonstrated by the large number of receptors that interact either directly or indirectly (via bridging molecules) with PS (46). However, nucleic acid receptors are also key mechanistic links in apoptotic cell-driven immune responses. The first solid evidence that innate recognition of mammalian DNA could drive immunity was the 2002 report by Ledbetter et al. demonstrating optimal chromatin-IgG complexes-driven activation of rheumatoid factor producing (i.e. anti-IgG) B cells required both B cell receptor crosslinking and MyD88/TLR9 dependent signals (58). This implied innate sensing of DNA is a key step in the generation of autoimmunity, promoting interferon production and inflammatory maturation of lymphocytes and antigen presenting cells. Overall, this concept fits neatly in the spectra of defects associated with systemic lupus erythematosus (SLE) which include reduced apoptotic cell clearance (59, 60), early development of IgG responses to chromatin and ribonucleoprotein complexes (RNPs) (61), and increased circulating chromatin immune complexes (62, 63). Moreover, genetic deletion of TLR9 reduces anti-DNA IgG titers (64) while deletion of TLR7 (an RNA binding TLR) reduces anti-RNP IgG titers in lupus-prone MRL^{lpr} mice (65). Thus, inflammation driven by specific categories of nucleic acids can similarly drive specific autoimmunity in an antigen and potentially cell-intrinsic manner. Likewise, elegant chromosomal mapping studies in the BXSB model of SLE demonstrated the lupus susceptibility locus Yaa is a duplication of the TLR7 gene (66). Functionally, Sylvia Bolland's group demonstrated TLR7 duplication in the Yaa loci significantly increased protein expression and function (67), which was responsible for the severe autoimmune pathology and distinctive autoantibody pattern targeting RNP structures in BXSB mice (68).

However, the contribution of TLR9 to apoptotic cell responses and BCR tolerance breakdown is somewhat less clear. In $MRL^{lpr/lpr}$ mice TLR9-signals provide a protective influence, as genetic deletion of TLR9 causes increases DC activation, interferon α production, and hypergammaglobulinemia (65). Deletion of the interferon α receptor 1 (abrogating responsiveness to IFNα/β) attenuates disease acceleration in $TLR9^{-/-}$ $MRL^{lpr/lpr}$ mice suggesting augmented type I IFN production is the mechanism driving pathology (69). Hence, TLR9 appears to have a dual role regulating as well as promoting of inflammatory autoimmunity. The contrasting functions may be explained by other signals intrinsic to DNA immune complexes. For example, high mobility group box 1 (HMGB1), a DNA binding protein that serves as an alarmin when released from activated neutrophils or necrotic cells (70), is required for full DNA/immune complex-driven activation of B cells (71). However, HMGB1 associated with apoptotic cells is tolerogenic due to modifications by caspase-

driven ROS burst and oxidation (72). Thus, like PS, the modification state of HMGB1 may be a critical determinant factor in the response to extracellular (or apoptotic cell exposed) DNA. In line with this concept, B cell exposure to apoptotic cells induces a TLR9-dependent, IL-10^{+} regulatory phenotype driven by recognition of DNA exposed in apoptotic blebs (49). Consequently, as a whole, the data indicates: I) multiple signals are required to augment TLR-driven responses to self-DNA, and II) DNA-dependent activation can have vastly different immunologic outcomes dependent on complex signal integration.

Surprisingly, cytoplasmic sensors of apoptotic cell DNA also appear to play a key role in apoptotic cell responses and immunologic outcomes. DNA-coupled to PEI nanoparticles (DNA nanoparticles, DNP) is a means of in vivo DNA transfection and gene delivery (73). It was reported by Dr. Andrew Mellor's group that DNPs carrying irrelevant DNA could induce profound immune suppression dependent on escape of the DNA from the phagosome into the cytosol with subsequent induction of type I IFN and downstream indoleamine 2,3 dioxygenase (IDO1)[i] (73). There are several cytosolic DNA receptors, however most (if not all) require the adaptor protein stimulator of interferon genes (STING) to induce type I IFN (74), thus STING provides a critical link between microbial signals and front line inflammatory immunity against cytosolic pathogen; paradoxically, however, STING was also required for DNP-driven regulatory responses inducing type I IFN production that lead to IDO activity (75). Shortly prior to publication of this observation, we had reported apoptotic cells induced IDO activity in MZ Mφs (76). Thus, we predicted STING would be involved in apoptotic cell-driven IDO activity. Supporting this hypothesis, STING$^{-/-}$ mice failed to induce IDO, IL-10, or TGF-β after apoptotic cell challenge (75). This finding was reinforced by recent evidence demonstrating STING deficiency accelerated disease in MRL$^{lpr/lpr}$ mice (77). We had noted previously that MRL$^{lpr/lpr}$ mice exhibited high levels of constitutive IDO expression that acted as a negative feedback mechanism retarding autoimmune disease progression (76). Sharma et al. found STING deletion results in a significant reduction of IDO protein in MRL$^{lpr/lpr}$ mice suggesting a functional relationship between efferocytosis, STING-dependent IFN production, and tolerogenic responses (77). STING initiates IFN expression by a TBK1 and IRF3-dependent signal-transduction pathway; however, the protective effect of STING on lupus progression appeared to be independent of IRF3 suggesting non-canonical STING signaling must be involved in the protective mechanism (77).

However, it is clear that STING activation by endogenous DNA can also drive inflammatory immunity. Deletion of the 3' exonuclease TREX-1 causes cell-autonomous activation of STING by accumulation of endogenous retroviral DNA products resulting in chronic type I IFN production and autoimmune disease (78, 79). Moreover pores formed in the mitochondrial membrane early during apoptosis can cause release of mtDNA inducing STING-dependent type I IFN production unless the DNA is deactivated by a caspase-dependent mechanism (80, 81). In these instances it is easy enough to explain the contrasting roles for STING, as recognition of DNA in this context is a cell-autonomous event. Thus exposure in this situation will have qualitative and quantitative differences

[i]There is also an IDO2 gene, however all work described herein refers to IDO1, thus use of IDO will refer to the IDO1 gene and protein hereafter.

compared to phagocyte recognition of apoptotic DNA, influencing immunologic outcomes of STING activation. It is more difficult to reconcile recent observations regarding apoptotic tumor uptake. In two reports it was observed that STING activation by tumor DNA drove type I IFN production and immune reactivity against potentially immunogenic tumors (82, 83). Yet in this situation, the STING-dependent effects were contingent on IRF3, suggesting a response distinct from the protective action of STING in $MRL^{lpr/lpr}$ mice (82). Moreover, a large proportion of tumor cells (perhaps as great as 90% for solid tumors) exhibit chromosomal instability (84) contributing to a high rate of spontaneous cell death (85). Thus the tumor environment may be analogous to observations in $DNAseII^{-/-}$ mice. In this case the significant accumulation of undigested apoptotic cells in macrophage lysosomes drives severe inflammation and embryonic lethality by activating STING (86, 87). This would suggest in the presence of a strong agonistic signal self-DNA may mimic infectious microbe DNA driving STING activation and a classic inflammatory phenotype in phagocytes; however, attenuated STING activation occurring in most cases of efferocytosis may promote limited IFN production that is sufficient to induce autocrine and paracrine IDO (and other regulatory mechanisms) but unable to drive pathologic changes.

Marginal zone cellular biology

B cells

With its connection to the circular system, the spleen has a unique role among the lymphoid organs. It functions as a circulatory filter, and as blood enters the spleen circulation flow slows allowing for capture of antigens from the blood, facilitating immune cell transport. In immune defense, the spleen is necessary for protection against encapsulated bacteria and in the absence of the spleen patients need medical intervention to prevent chronic, severe bacterial infection (88–90). The spleen is organized in a red and a white pulp with the marginal zone (MZ) situated between these. In mice the white pulp follicle is further divided in B cell and T cell areas that resemble the structure of a lymph node. B cells in the different zones are named after their micro-anatomical location, i.e. Marginal zone B cells (MZB cells) and Follicular B cells respectively (91, 92). These two B cell subsets are in majority in the spleen with MZB cells constituting between 5–10% of the total B cells in mice. Also, in the spleen a small number of B1 B cells can be found, with a dominance of the B1a subtype over B1b; however, it is not known if B1 B cells are recruited from other areas of the body or are enriched in a specific niche (93). In mice blood entering from the splenic artery terminates at the marginal sinus, which is a low resistance vessel that allow blood to flow through the marginal zone into the red pulp, and subsequently to the venous sinuses and back into venous circulation. In humans there is no marginal sinus but instead the blood entering from the splenic artery flow through penicillary arterioles into the red pulp and perifollicular zone with and open structure (94). Architectural differences between mouse and human spleen are discussed below.

MZB cells

Development of MZB cells occurs after birth and is connected to colonization of bacteria. One piece of evidence for this demonstrates gut-associated lymphoid tissue (GALT) is needed for their differentiation in rabbits. (95). In mice, MZB cells derive from transitional

B cells (T1) belonging to the B-2 lineage arriving to the spleen from the circulation. After T1 B cells enter the spleen, they pass through a peripheral checkpoint that removes a portion of the self-reactive pool (96, 97). After this they go on to differentiate into transitional type 2 (T2) B cells and are subsequently selected into the MZB cell and follicular B cell populations. The decision on which pool to enter depends on signaling strength from the BCR and the downstream signaling molecule Btk (92). NOTCH2 signals are key in MZB cell development and engagement of NOTCH2 by the ligand DLL on endothelial cells drives development of MZBs (98). NOTCH2 has also been linked to BCR signaling but there is also data suggesting NOTCH2 regulates MZB development in absence of the BCR (99). The mature MZBs differentiate into a B cell that expresses high levels of IgM, CD1d and a number of other markers including CD9 and CD36. There is an overlap in the BCR repertoire with FOBs but the MZB pool is enriched for reactivity towards modified self-antigens such as phosphatidylcholine (PC) (100).

Since the human and mouse spleen architecture are very different (as described below), and the markers on B cells do not overlap completely, defining the human equivalent to mouse MZBs has proven difficult. One significant point is the fact that IgM^{high}/IgD^{low} cells found in the marginal zone in humans (unlike mice) posses mutated B cell receptors, the memory marker CD27, and are thus considered to be a product of the germinal center (101). Recently however, the fact that mouse MZBs need NOTCH2 signaling for their development was used to define a similar human population of B cells (102). This population can be found in blood and spleen and are $IgM^{+}IgD^{+}CD27^{+}$ but differentiated from switched B cells by altered expression of a number of genes including Sox7 (102). B cells with the $IgM^{+}IgD^{+}CD27^{+}$ phenotype also develop in individuals with hyper-IgM syndrome showing that they can develop in absence of a germinal center reaction (103). Instead, the diversification via hyper-mutation of the innate type B cell pool including MZBs are closely linked to GALT (104). Interestingly, the innate pool of human B cells including MZBs is fairly large and appears more diverse than in mice. Thus, at the moment it is unclear if the human pool of innate B cells can be subdivided into separate innate lineages including an equivalent of mouse B1 B cells (105).

An important function of MZBs is to transport Immune complexes to the follicle (106). To exert this function they highly express a number of receptors that can capture antigens in the form of immune complexes (FcgRIIB) or antigens that activated complement and are opsonized (CD21) (107, 108). Complement activation in the MZ itself is also facilitated by the fact that MZ Mφ express SIGN-RI that can activate complement locally. In addition, they are also enriched for other pattern recognition receptors, including TLR7, CD36 and tetraspanin CD9 (109–111). In addition to this, MZBs are larger and have higher levels of MHC Class II as well as costimulatory molecules needed to differentiate T cells to the Tfh phenotype. This fact paired with that they are able to directly differentiate into plasma cells makes MZBs a versatile cell, capable of transport of antigen, quickly induce antibody production as well as support T cells needed for the germinal center reaction.

Stroma cells, granulocytes and innate lymphocytes

The backbone for the compartmentalization of the different areas is the specialized stromal cells that provide structure and chemotatic signals driving immune cell migration and activation (112). Marginal reticular cells (MRCs) are localized primarily to the MZ, although they can also be found at the outer follicular regions of several secondary lymphoid organs (113). These are distinct from fibroblast reticular cells (FRCs) and follicular dendritic cells (FDCs) that drive formation and structural organization of the white pulp. In the white pulp, FRCs make up a network of tubular network conduits for structure and transport of antigens, and they are also a key cellular producer of homeostatic levels of BAFF. Thus FRCs are critical for establishment of a favorable niche for B cells and their compartmentalization in the spleen (114). Follicular FDCs have the unique ability to capture and recirculate antigen for B cell activation making them a key cellular component of both initiation of the humoral response and B cell selection in the germinal center (115). In the red pulp endothelial cells lining the venous sinuses govern blood flow and screen blood for aged erythrocytes caught in the splenic cords. These will subsequently be removed by red pulp macrophages that align the sinuses (91). The red pulp also harbors a reservoir of monocytes that are mobilized by systemic inflammation and can be rapidly recruited to sites of injury (116). During tumorigenesis, this niche is inhabited by an increased pool of cells with myeloid and neutrophil markers, so called Myeloid derived suppressor cells (MDSCs), that suppress T cell responses and are precursors of tumor associated macrophages that promote tumor growth (117).

In addition, the red pulp and MZ is populated by neutrophils that aid in MZB activation by expression of TNF-family members, BAFF and APRIL as well as IL-21 (118). It has also been shown that the MRCs in the marginal zone interact with innate lymphoid cells (ILCs) regulating their function through TNF and lymphotoxin production (119). ILCs are potent producers of cytokines and their secretion pattern and development mirror that of T cells although they do not express antigen receptors (120). Magri et al. demonstrated that the ILCs near the MZ supported MZB cell survival and function by a mechanism dependent on expression of BAFF and the NOTCH ligand delta-like 1 (DLL1) as well as CD40L-dependent stimulation (119). Interestingly, DLL1 together with BAFF is needed for MZB cell development (98), so it is possible that ILCs in the spleen aid in MZB cell development as well as activation.

Macrophages

An integral component of the splenic stroma is the unique resident Mφ populations that reside in the red and white pulp (121). In the white pulp, both the T and B cell zones house distinct Mφ populations. Of these, the function of tingible body Mφs (TBMs) is the most extensively studied. These Mφs have a key mechanistic role in the germinal center as they are the primary phagocyte responsible for clearing apoptotic B cells (hence the terminology, as the “tingible bodies” are remnants of apoptotic nuclei in the Mφs). In the germinal center PS recognition is a key signal for clearance, primarily via indirect mechanisms involving bridging molecules. For example, mice lacking the TAM receptor MER exhibit accumulated, non-phagocytized apoptotic cells on the surface of TBMs (122). TBMs also show an extensive presence of MFG-E8 on the cell surface; although TBMs do not express

MFG-E8, which is produced by nearby FDCs in the germinal center (123). Thus, through this production of soluble opsonins, FDCs may partially "license" TBM for efferocytosis (16).

In the red pulp a large number of Mφ can be found associated with the FRCs, governing red blood cell turnover and iron recycling (124). In and around the MZ, there are at least two distinct Mφ populations, Marginal zone Mφ (MZ Mφ) which isolate as a band in the MZ proper, and metallophilic macrophages (MMφ) that exist as a thin band of cells in the follicle directly underneath the MAdCAM-1^+ cells lining the marginal sinus. The MMφs have a phenotype similar to subcapsular Mφs in the lymph node and most likely exhibit similar function in which they capture and transfer antigens in circulation (either blood or lymph) to be transported to the FDCs (121). In addition they have high levels of enzymatic activity and can transport antigens to the follicular FRC conduit network where antigens may be presented on FDCs or DCs in the PALS (125). MZ Mφs, on the other hand, are in close physical contact with the MZB cells and together the two cell populations create a potent cellular mechanism for capture of antigens including immune complexes, systemic pathogens, and self-antigens, (including oxidized LDL and apoptotic cells) (108, 126, 127). The close physical and mechanistic association of MZ Mφs and MZB cells is required for function, development and differentiation of both cell populations (128, 129). Some of the specific receptors expressed by MZ Mφs also aid in the capture and response to antigens captured here, exemplified by SIGN-RI that has the ability to activate complement (130).

NKT cells

In the MZ and red pulp there is a population of NKT cells expressing T cell receptors that can recognize glycolipids presented on the MHC I like molecule CD1d (62). MZB cells express very high levels of CD1d and it has been shown that they interact with NKT cells with either inflammatory or suppressive outcomes depending on the glycolipids activating the NKT cells (63, 64). Moreover, when responding to foreign glycolipids, NKT cells take on a helper phenotype (NKTfh) in a manner similar to T follicular helper cells, but unlike T cells the activation of B cells via NKTfh cells does not result in long lived plasma cells instead driving IL-10 producing regulatory B cells (131). NKT cells are potently activated by glycolipid antigens of self-origin including glycosphingolipids (GSL) and phospholipids like lysophosphatidylcholine (lysoPC) (66). However, the exact extent of NKT cell self-reactivity not known, and self-lipids involved in their selection in thymus are likely different from the ones generated during inflammation.

NKT cell regulation of B cell responses when they are activated by foreign glycolipids presented in CD1d can be divided into cognate vs non-cognate interactions (132). During the non-cognate activation NKT cells promote B cell responses trough interactions with CD1d expressing DCs that in turn drive T cell and B cell responses. This interaction drives antibody production and memory formation. This is separate from cognate interaction between NKT cells and B cells that promote antibody production without memory. Instead this interaction gives rise to IL-10 producing regulatory B cells that differentiate to plasma cells producing antigen-specific and poly-reactive IgM and IgG (133, 134). In this way, regulatory B cells can limit a specific immune response and also aid in clearance of the

antigen. This regulation is in contrast to NKT cell regulation of B cell responses during sterile inflammation or after exposure to apoptotic cells (135, 136). In both circumstances, NKT cell deficient mice exhibit much stronger immunity compared to wild type controls. In the case of apoptotic cells, mice also develop signs of disease with involvement of kidney pathology (136). The self-glycolipid being presented on CD1d in these models is not known and could be derived from apoptotic cells as a result of alterations of the membrane or be an endogenously produced ligand. CD1d is always occupied with a glycolipid and it has been shown that endogenously produced glucosylceramides can be produced by mammalian cells (137) suggesting homeostatic activation of NKT cells. However, how the enzyme systems driving self-lipid production are tightly regulated and if they respond to signals of cell death is not known. In inflammasome-driven responses, NKT cells may suppresses inflammatory activity by perforin or FAS-dependent killing of inflammatory effector cells (135). This capacity has been shown to be involved in inflammatory diseases such as hypersensitivity or atherosclerosis (138, 139). NKT cell induced killing has been shown to correlate with CD1d expression and thus NKT cells are most potent in killing MZBs as shown in the paper by Wingender and colleagues (140). The involvement of NKT cells in regulating tolerance is further strengthened by the findings that $CD1d^{-/-}$ mice were unable to develop tolerance to OVA in the anterior chamber associated immune deviation (ACAID) model of systemic tolerance (141). This is a model for tolerance induction at immune-privileged sites that relies on the spleen, with involvement of macrophages and tolerance imposed on B cells. However, how NKT cells are polarized in this model, and if there is also activation of IL-10 producing regulatory B cells has not been investigated.

Human versus mouse

When applying mechanistic observations of marginal zone function in mouse or rat models to humans, consideration must be given to anatomical differences. As described above, in mice there is a highly ordered compartmentalization of the spleen with the marginal sinus providing a clear demarcation between the red pulp and the majority of the white pulp follicles (91). Moreover, in the mouse marginal zone and metallophillic Mφ populations are readily identifiable based on expression of MARCO and SIGN-R1 (MZ Mφs) and CD169 (sialoadhesin/Siglec-1, MMφs). Rats have a splenic MZ architecture comparable to mice, although CD169 is expressed on MZ Mφs as well as MMφs suggestive of potential diversification of function (142). Furthermore, in rodents the branching splenic arterial vessels are extensively sheathed by lymphocytes in a predictable and consistent fashion that, at least for mice, appears to have little variation between common laboratory strains (TLM, unpublished observations). In humans, splenic cellular architecture and function is still a subject of debate. While possessing clearly demarcated white and red pulp regions, human spleen lacks a marginal sinus and a functionally demarcated marginal zone (143). Rather, the region surrounding the germinal center follicle consists of a follicular dendritic cell (FDC)-supported mantle zone containing $IgM^{+}IgD^{+}CD20^{+}$ B cells (144) with an adjacent superficial zone. The superficial zone contains specialized $MAdCAM\text{-}1^{+}CD90^{+}CD106^{+}$ fibroblasts that may aid in lymphocyte migration from the red pulp to the PALS (145). Human spleen also contains an extra compartment not found rodents, the perifollicular zone (PFZ). The PFZ, located between the superficial zone and the red pulp, is notable for the presence of numerous capillaries sheathed by $CD68^{+}CD169^{+}$ Mφ (145, 146). The outer

edge of the PFZ is continuous with red pulp sinuses suggesting it is a specialized extension of the red pulp (145, 147, 148).

Comparatively, circulation dynamics are distinct as blood enters the human spleen via terminating capillaries that empty in to the splenic cords (149) while in rodents much of the blood enters via the marginal sinus (91). This raises questions regarding the sequence of events leading from apoptotic cell capture to long-term immunologic tolerance that have yet to be resolved. In the end it is likely sheathed capillary Mφ in the spleen are a functional equivalent of MARCO+ Mφ in the mouse MZ capturing particulates in the circulation (including apoptotic cells). Supporting the idea of mechanistic convergence is the observation that i/v apoptotic cell infusion drives Treg expansion (150, 151), improves allograft survival and function in a variety of transplant settings (152–154), and reduces autoimmune T cell responses (155) recapitulating observations in mice following apoptotic cell infusion (151, 156, 157).

Apoptotic cell response in the

Macrophages

MZ Mφs and metallophillic CD169$^+$ Mφ (MMφs) share a close developmental and functional relationship. In particular developmental defects or depletion strategies lead to the absence of both populations of Mφs. Moreover, mice deficient in MZ B cells are also deficient in MZ Mφ and MMφ and restoration of the MZB population restores both Mφ populations (13). This shows constant cell-cell communication is required to maintain the MZ cellular architecture under steady-state conditions. This also creates a challenge since it is difficult (with current tools) to determine cell-specific mechanisms after depletion or congenital deficiency. Nevertheless, by isolating MZ Mφs and MMφs after apoptotic cell exposure a picture has begun to emerge suggesting distinct, non-overlapping function for these Mφ populations in apoptotic cell-driven tolerance.

It has been known for some time that MZ Mφ are critical for particulate trapping in the spleen. We have found particles with a size of 1μM are efficiently trapped in the MZ in close contact with MZ Mφs (158), while nanoparticles (>100nM is size) are captured less efficiently diffusing into the red pulp where they interact to a greater degree with other phagocytes (75). When the spleen is depleted of MZ Mφs, overall apoptotic cell clearance from circulation is not impacted in a significant fashion; but, apoptotic cell trapping in the MZ is lost and the cell debris is instead dispersed throughout the red pulp with aggregate accumulation in the white pulp (158). This change in phagocytic localization is associated with a significant increase in efferocytosis by CD68$^+$F4/80$^+$ red pulp Mφs and CD11b$^+$ DCs (158, 159). There is also a dysregulation of apoptotic cell responses by phagocytes as efferocytosis drives acquisition an inflammatory phenotype with induction of TNF-α, IL-6, and IL-12p40, upregulation of MHCII and CD86, and CCR7-dependent migration to the white pulp (158). Moreover, CD8α$^+$CD103$^+$ DC that drive adaptive tolerance to apoptotic cells (151, 160) appear to have reduced efferocytosis function (159). Ultimately, this change in dynamics of the apoptotic cell response promotes a microenvironment conducive to inflammatory adaptive immunity, and we found that chronic exposure to apoptotic cells in MZ Mφ-depleted animals drives systemic and eventually fatal autoimmunity (158).

Gene expression analysis on sorted phagocytes after efferocytosis indicated apoptotic cells drive a distinctive cytokine signature in MARCO$^+$ Mφ that includes prominent induction of type I IFNs (TLM, unpublished observations). In general IFN stimulated genes (ISG) are associated with inflammatory immunity, however the IDO promoter contains several gamma-activated sequences (GAS) and interferon stimulation responsive elements (ISRE) making the IDO gene sensitive to type I type II IFN stimulation (161, 162). Thus IDO is one of a handful of ISG that has an unambiguous a regulatory function, limiting IFN-driven inflammation. As mentioned earlier, splenic IDO is induced by apoptotic cell exposure i/v (76); moreover, expression segregates with the IFN signature in MZ Mφs, and MZ Mφ depletion abrogates IDO induction after apoptotic cell exposure (76). Importantly, when IDO activity is antagonized, splenic responses to apoptotic cells phenocopies MZ Mφ depleted mice with an attenuation of IL-10 and TGF-β induction and concomitant proinflammatory cytokine synthesis and T cell responses to apoptotic cell antigens (76). This suggests IDO serves a key role in regulation of the innate response to apoptotic material and ensuing apoptotic cell-driven immune suppression.

IDO is an intracellular heme-oxygenase that targets indole ring-containing compounds including the essential amino acid tryptophan (163). Although IDO can regulate immunity by production of tryptophan metabolites (163), it appears that IDO induction in MZ Mφ regulates local dendritic cell and macrophage responses to apoptotic cells by consumption of micro-environmental tryptophan driving the general control nonderepressible 2 (GCN2) arm of the integrated stress response (157). GCN2 is a Ser/Thr kinase that binds uncharged tRNAs that accumulate when intracellular amino acid pools are depleted (164). Activated GCN2 phosphorylates the translation factor eIF2α altering ribosomal assembly dynamics and protein translation (165). In myeloid cells, efferocytosis suppresses IL-12 promoter activity (166), and GCN2 activation by IDO augments this effect by inhibiting IL-12 translation; this is in contrast to IL-10, which is enhanced transcriptionally by efferocytosis but not impacted at the translational level by activation of GCN2 (157). However, when GCN2 is deleted IL-12 mRNA association with polyribosomes is not inhibited by apoptotic cells; thus promoting IL-12 synthesis that has a feedback effect inhibiting production of IL-10. This explains the phenotype we have observed in IDO-inhibited mice, and suggest apoptotic cells are capable of stimulating inflammatory immunity that must be counteracted to prevent inflammatory responses.

When exposed to apoptotic cells, MMφs rapidly induce expression of the chemokine CCL22 (151). CCL22, also known as macrophage-derived chemokine (MDC) (167), is a T cell chemo-attractant that interacts with the cognate receptor CCR4 (168–171). CCR4 has a predominately lineage specific expression on Th2 T cells and a subpopulation of Foxp3$^+$ regulatory T cells, and expression of CCL22 drives Treg accumulation and suppression of immunity (170, 172–174). Similarly, CCL22 induction by MMφs drives significant accumulation of Tregs in the splenic white pulp within hours of exposure (151). DC can also express CCR4 under certain circumstances (175), and CCL22 expression by MMφ promoted accumulation of CCR4$^+$CD103$^+$CD8α$^+$ tolerogenic DCs in the spleen (151). This significantly increased Treg-DC interactions in the white pulp promoting downstream suppressive immunity. Importantly, inhibition of either CCL22-driven chemotaxis or Treg function was sufficient to block DC tolerogenic maturation or apoptotic cell-driven tolerance

(151). A similar role for splenic MMφs has been described in protection from ischemic injury (176) suggesting a key role for MMφs in coordinated regulation of cellular migration and promotion of down stream adaptive tolerance.

Dendritic Cells

The spleen contains several DC populations with distinct localization patterns and functional segregation. The major $CD11c^+$ populations can be differentiated on the basis of CD8α expression while plasmacytoid dendritic cells are $B220^+PDCA^+SiglecH^+CD11c^{low}$ (177). $CD8\alpha^{neg}$ DCs localize primarily outside the white pulp and are thus more abundant in the MZ and red pulp while $CD8\alpha^+$ DCs have a more generalized distribution and are found in both the red and white pulp. Clearly $CD11c^+$ DC play a key mechanistic role in the maintenance of peripheral tolerance since ablation using a CD11c human diphtheria toxin receptor transgenic model resulted in rapidly fatal autoimmune disease (178). Surprisingly, abrogation of DC apoptosis leads to the development of systemic autoimmunity suggesting DCs apoptosis may also be a key factor in maintaining the immune rheostat (179). Our data suggest cognate interaction between DCs and Tregs is an important instructive event promoting tolerogenic DC maturation (151). Likewise, Treg functional activity is dependent on signals delivered by the DCs (180). This would insinuate constitutive interactions between DCs and Tregs in the context of self-antigen are required for the maintenance of suppressive DC and Treg identity and downstream tolerance, an idea consistent with the finding that Treg suppression of autoimmunity requires the constant presence of self-antigen (181).

It is generally believed efferocytosis leading to adaptive tolerance requires a population of $CD8\alpha^+CD103^+CD11c^+$ DCs found in the MZ and red pulp. These DCs preferentially capture apoptotic cells in a variety of circumstances and are potent drivers of suppression (151, 182–185). In the spleen, $CD103^+$ DC are functionally distinct from $CD8\alpha^+CD103^{neg}$ DCs as they exhibit a profound defect in IL-6 production after LPS activation (185). Moreover, they appear specialized to capture apoptotic cells after which they migrate to the PALS where they may present antigens to T cells, tolerizing inflammatory responses (185). Supporting this idea, deletion of $CD103^+$ splenic DCs significantly attenuates apoptotic cell driven suppression of inflammatory autoimmune disease (185).

$CD8^+$ DCs provide the link between innate efferocytosis and adaptive T cell tolerogenic mechanisms, and they cross-present apoptotic cell antigens potently suppressing both $CD4^+$ and $CD8^+$ autoimmune T cell effector responses (183, 184, 186, 187). Cross presentation function is a constitutive activity in $CD8\alpha^+$ DCs and likely not apoptotic cell-specific per se (186, 187). However, an essential, apoptotic cell-driven feature of long-term tolerance is Treg expansion from the naïve repertoire (156). Expansion is self-antigen driven, and our recent data suggests autoreactivity in the preformed (i.e. natural) nTreg population in the periphery is required for initiation of the process (151). Based on these observations, a case can be made for autoimmune Treg-driven priming of apoptotic cell tolerance since apoptotic cell challenge rapidly induced surface CTLA-4 on splenic Tregs suggestive of apoptotic cell-induced activation prior to differentiation of naïve T cells (151). Treg expansion requires induction of TGF-β, and low antigen availability (188). Our laboratory has found

that apoptotic cell challenge induces prominent TGF-β production in a segregated fashion in $CD8\alpha^+$ DCs in the spleen (151, 157). Moreover, it would be predicted antigen load for any apoptotic cell associated molecule would be low. Likewise cellular particulates are efficient at directing antigens towards MHCII presentation in a mechanism of tolerance consistent with the notion of DC directed $FoxP3^+$ Treg generation (189). Together this suggests the $CD103^+CD8\alpha^+$ DC population is the one primarily responsible for apoptotic cell-driven Treg induction.

PDCs have massive ability to produce type I IFN, and their expansion and activation are closely associated with systemic autoimmune disease progression in both mouse and man. However PDC exposure to apoptotic cell-released microparticles drives IFNα production in a mechanism dependent on TLR9-driven recognition of apoptotic cell DNA (190). Further, Phillip Sass's group found that apoptotic cell infusion i/v drove Treg expansion in a mechanism dependent on Mφ/PDC cross talk (191). This suggests PDCs may play a previously unknown role in establishment of the immune regulatory network following efferoctyosis. As such, PDCs can induce suppressive immunity by variety of mechanisms including IL-10 secretion, expression of IDO, and upregulation of PD-L1, and ICOS-L (192–194). However, we have not observed a significant physiologic response in splenic $CD11c^{low}B220^+$ PDCs following apoptotic cell exposure (TLM, unpublished data). Nevertheless, this does not exclude a role in apoptotic responses under other circumstances.

Natural Immunoglobulin

Natural antibodies are defined as inherited and germline encoded antibodies produced by naïve mature B cells that have gone through selection but that are unaffected by external antigenic stimuli (195). The discovery of natural immunoglobulin stems from studies of antibodies found in germ-free mice as well in human cord blood (196). Determining the cellular source, experiments in mice have shown that typically 90% of the circulating natural IgM is derived from B1 B cells (197). A portion of the B1 B cells produce antibody that can recognize the head group of modified phosphorylcholine (i.e. PC) generated during apoptosis (198). This includes T15, a high affinity IgM B cell clone repeatedly identified in screens for reactivity to PC (199). Originally, interest in T15 was directed to its importance in protection from pneumococcal infection (200). However, T15 dominates the early life anti-PC response, even in germ free mice (201, 202). Given the cross-reactivity to self-lipids, one explanation for the dominance of T15 is endogenous positive selection in response to apoptotic cells. T15 can bind directly, and specifically, to apoptotic cells (203) and will likely aid in clearance by complement dependent mechanisms. However, T15 also has a direct inhibitory effect on phagocytes driving induction of the negative regulatory of MAPK signaling, MAPK phosphatase 1 (204). This regulation occurred either in the presence of C1q mannose binding lectin suggesting T15-ellicits multiple regulatory signals (204). Moreover, apoptotic cell exposure rapidly induces IgM responses to PS, PC and the lipid neo-antigen malondialdehyde (MDA), including T15 (205). Although much remains to be determined, it is clear innate (i.e. rapid, non-mutated) IgM responses to apoptotic cells are a critical component of splenic immunity to self.

Complement and clearance

The complement system is a major component of innate immunity, consisting of over 40 proteins of which 25 are soluble and produced mostly by the liver (206). However, myeloid cells can produce many of the proteins locally during an immune response as an integrated part of innate immunity (207). Over a century of research has shown that the complement system is an important part of the immune system and its activation is mediated through three major pathways (The classical, lectin, and alternative pathways) (207, 208). The classical pathway involves antibodies and is activated when the C1q protein binds to its ligands, including immune complexes containing sIgM as well as sIgG (209). The binding of C1q is restricted to pentameric sIgM whereas it has no affinity for the monomeric protein. On the other hand, a single pentameric sIgM molecule is enough to activate the complement cascade in *in vitro* assays, making it 1000 more efficient than sIgG (210, 211). Complement factors and especially C1q are involved in the removal of dead cells and cellular debris, and by this can take part in tissue homeostasis (212). In the marginal zone local complement activation is further facilitated by MZ Mφs expressing SIGN-RI, a receptor shown to be able to mediate complement fixation (130). The importance of complement-mediated clearance is best illustrated by the fact that over 90% of patients with C1q deficiency develop SLE, and C1q deficient mice develop spontaneous lupus-like disease (15, 57). These data show that the complement system plays an important role in the tolerogenic perception of apoptotic cells. Mechanistically, it has been shown that C1q coated apoptotic cells suppress inflammation by induction of IFNs, IL-27 and IL-10 in macrophages (213). This response will in turn suppress Th1 and Th17 activation of T cells and thus further suppress the pro-inflammatory response (214). Unlike apoptotic cells, live cells are protected from complement attack by regulatory molecules on their surface, including factor H which binds to C3b which is a cleavage product of C3. The end result is conversion of C3b to iC3b, which also participates in clearance of apoptotic cells via CR3 on macrophages and dendritic cells in the MZ. This iC3b-CR3 interaction drives immune suppression by down regulation of co-stimulatory molecules, inhibition of maturation, and antagonizing production of pro-inflammatory cytokines including IL-12 (54, 215). These pathways are critical to regulate complement activation, as C3 can also be a very potent molecular adjuvant when opsonizing in the form of C3d. This was elegantly shown in a seminal paper where tagging of one or more cleavage products of C3 (C3d) enhanced the response in a stepwise fashion to the protein antigen hen egg lysozyme (HEL) up to 10 000 times (216). MZBs also transport C3d tagged antigen or immune complexes to the follicle for deposition on FDCs (217). Thus inappropriate clearance and regulation via complement could drive chronic activation and deposition of self-antigen on FDCs (218). This, together with the fact that the MZ is enriched for scavenger receptors that bind apoptotic cells, makes this region key in the regulation of tolerance towards self-antigens (219). The protective scavenging function also extend to atherosclerosis where secreted (s) IgM and C1q-deficient mice exhibit an enhance disease when crossed to atherosclerosis prone LDL receptor-deficient mice (220). The disease in mice lacking sIgM was more severe than in mice lacking C1q suggesting that protection is only partly regulated by the classical pathway of complement activation.

MZ defects and contribution to autoimmune disease

Defects in recognition, clearance, or destruction of apoptotic cells is closely linked with the development of systemic autoimmune disease in mouse and man. In SLE the earliest and primary antigens targeted are exposed on apoptotic blebs (50, 221) suggesting defective homeostatic processing of apoptotic cells leads to inflammatory responses to these widely available auto-antigens. Exactly what defects drive abnormal apoptotic cell responses are not clear as there is no significant association between SLE and the major apoptotic cell recognition machinery, with the notable exception of C1q. While human splenic structure does not contain a marginal zone, recent lessons learned in the mouse MZ have applicability to general defects in phagocyte function and their contribution to disease activity in SLE. For example, Birjandi et al found aged (i.e. 18 months old) BALB/c mice showed altered MZ cellularity including significantly decreased and disorganized MZ Mφs and a reduction of MZB cells (222). Moreover, there was a reduction and reorientation of the MAdCAM-1$^+$ cells lining the marginal sinus towards the white pulp in a discontinuous distribution (222). The MAdCAM-1$^+$ cells form a barrier to follicular access and regulates lymphocyte homing (223–225), thus aging may compromise the ability of the MZ to regulate follicular access.

BXD2 mice are a lupus prone strain of derived from a cross of C57BL/6 and DBA/2. Li et al. reported as BXD2 mice age and progress to autoimmunity, there is a progressive defect in MZ Mφ numbers (226). Importantly, as MZ Mφ numbers decreased MZB cells transporting apoptotic cell debris showed increased follicular access (226). This resulted in T cell activation and inflammatory cytokine production. Loss of MZ Mφs was also associated with a decrease in IDO expression and the negative regulator of signal transduction SHIP1 (226). Importantly, spleens from SLE patients showed a similar loss of MARCO+ Mφs (227). Mechanistically, the loss of MZ Mφs was the result of IFN-driven repositioning of membrane lymphotoxin-α1β2$^+$ MZB cells from the MZ to the follicle. The loss of lymphotoxin-α1β2 signaling in MZ Mφs reduced expression of the transcription factor megakaryoblastic leukemia 1 (MLK1) which attenuated actin expression, driving decreased efferocytosis and a gradual loss of MZ Mφs (227). This important finding suggests chronic IFN production can compound autoimmunity by compromising the ability of the spleen to properly capture and dispose of apoptotic debris.

Apoptotic cells and atherosclerosis

There is an overlap in the reactivity between apoptotic cells and oxidized LDL (oxLDL) particles as both modified self-antigens contain oxidation specific epitopes that can be recognized by the same antibodies (228). This reactivity is also present in the natural antibody pool, and serum from newborns bind both apoptotic cells and atherosclerotic plaques (229). In atherosclerosis cholesterol in the vessel wall generates oxLDL which drives vascular inflammation promoting pathology, but recently it was shown that this accumulation (including the formation of cholesterol crystals driving inflammasome activation) could also occur in the spleen (126). The antibody reactivity towards oxLDL includes T15, which may be key in oxLDL clearance (203). B cells have a dual role in atherosclerosis as they can also add to pathology enhancing inflammation and driving T cell responses (230, 231). On the other hand, natural IgM and PC-specific antibodies are known to be protective. A clear example of this is the observation that transfer of B cells but not T

cells from atherogenic mice provides protection from atherosclerosis (232). This was later shown to be due to transfer of an ongoing innate inflammatory B cell response that included production of T15 antibodies (126). Interestingly, injecting mice with apoptotic cells could induce the same arthero-protective response. This shows that the response to apoptotic cells in the marginal zone of the spleen have a buffering effect by the induction of a natural antibody response as well as some of the other mechanisms discussed earlier. This links regulation in the MZ to atherosclerosis and may explain studies linking splenectomy, altered cholesterol levels, and heart disease in humans (233, 234).

Conclusions

It is clear that the spleen in general, and the marginal zone in particular, plays a key role in the homeostasis related to buffering of systemic inflammation to apoptotic cell death. This system is closely connected to lipid metabolism and responses are evoked by a number of systems of pattern recognition that overlap with the response to foreign antigens. However, the complex in vivo mechanisms driving apoptotic cell suppression remain, generally speaking, poorly understood. Since many autoimmune and inflammatory diseases are a result of, or result in, imbalance in reticuloendothelial clearance apoptotic cells, targeting the machinery involved in immune homeostasis to apoptotic cells may be an attractive approach to modulate the tolerogenic rheostat. However, important questions remain regarding the general applicability of mechanisms described in the mouse marginal zone to human disease as well as the characteristics of stimuli that drive initial and long term immune suppression. From a therapeutic perspective it will be key to determine if is there a specific set of canonical signals that give rise to the apoptotic cell suppressive response that could be incorporated in a tolerogenic vaccine, or conversely blocked for improved traditional vaccine efficacy.

Acknowledgements

This work was supported by NIH grants R01AR067763, R01CA190449, and R21AI105500, the lupus research institute (TLM), the Swedish Research Council, the Magnus Bergvall Foundation, the Swedish Medical Society, the Swedish Rheumatic foundation, the King Gustav V 80-year Foundation, the Torsten Söderberg foundation, and the Cardiovascular Research Programme (MCIK).

References

1. Majno G, Joris I. Apoptosis, oncosis, and necrosis. An overview of cell death. The American journal of pathology. 1995; 146:3–15. [PubMed: 7856735]
2. Kerr JF, Wyllie AH, Currie AR. Apoptosis: a basic biological phenomenon with wide-ranging implications in tissue kinetics. British journal of cancer. 1972; 26:239–257. [PubMed: 4561027]
3. Kerr JF. Shrinkage necrosis: a distinct mode of cellular death. The Journal of pathology. 1971; 105:13–20. [PubMed: 4108566]
4. Saunders JW Jr, Gasseling MT. Cellular death in morphogenesis of the avian wing. Developmental biology. 1962; 5:147–178. [PubMed: 14497532]
5. Silva MT. Secondary necrosis: the natural outcome of the complete apoptotic program. FEBS letters. 2010; 584:4491–4499. [PubMed: 20974143]
6. Bratton DL, Henson PM. Neutrophil clearance: when the party is over, clean-up begins. Trends Immunol. 2011; 32:350–357. [PubMed: 21782511]

7. Voll RE, Herrmann M, Roth EA, Stach C, Kalden JR, Girkontaite I. Immunosuppressive effects of apoptotic cells. Nature. 1997; 390:350–351. [PubMed: 9389474]
8. Fadok VA, Bratton DL, Konowal A, Freed PW, Westcott JY, Henson PM. Macrophages that have ingested apoptotic cells in vitro inhibit proinflammatory cytokine production through autocrine/paracrine mechanisms involving TGF-beta, PGE2, and PAF. J Clin Invest. 1998; 101:890–898. [PubMed: 9466984]
9. Fadok VA, Voelker DR, Campbell PA, Cohen JJ, Bratton DL, Henson PM. Exposure of phosphatidylserine on the surface of apoptotic lymphocytes triggers specific recognition and removal by macrophages. J Immunol. 1992; 148:2207–2216. [PubMed: 1545126]
10. Fadok VA, Bratton DL, Rose DM, Pearson A, Ezekewitz RA, Henson PM. A receptor for phosphatidylserine-specific clearance of apoptotic cells. Nature. 2000; 405:85–90. [PubMed: 10811223]
11. Hoffmann PR, et al. Phosphatidylserine (PS) induces PS receptor-mediated macropinocytosis and promotes clearance of apoptotic cells. J Cell Biol. 2001; 155:649–659. [PubMed: 11706053]
12. Platt N, Suzuki H, Kodama T, Gordon S. Apoptotic thymocyte clearance in scavenger receptor class A-deficient mice is apparently normal. J Immunol. 2000; 164:4861–4867. [PubMed: 10779795]
13. You Y, Zhao H, Wang Y, Carter RH. Cutting edge: Primary and secondary effects of CD19 deficiency on cells of the marginal zone. J Immunol. 2009; 182:7343–7347. [PubMed: 19494255]
14. Cohen PL, et al. Delayed apoptotic cell clearance and lupus-like autoimmunity in mice lacking the c-mer membrane tyrosine kinase. J Exp Med. 2002; 196:135–140. [PubMed: 12093878]
15. Botto M, et al. Homozygous C1q deficiency causes glomerulonephritis associated with multiple apoptotic bodies. Nature genetics. 1998; 19:56–59. [PubMed: 9590289]
16. Hanayama R, et al. Autoimmune disease and impaired uptake of apoptotic cells in MFG-E8-deficient mice. Science. 2004; 304:1147–1150. [PubMed: 15155946]
17. Hoeppner DJ, Hengartner MO, Schnabel R. Engulfment genes cooperate with ced-3 to promote cell death in Caenorhabditis elegans. Nature. 2001; 412:202–206. [PubMed: 11449279]
18. Reddien PW, Cameron S, Horvitz HR. Phagocytosis promotes programmed cell death in C. elegans. Nature. 2001; 412:198–202. [PubMed: 11449278]
19. Lauber K, et al. Apoptotic cells induce migration of phagocytes via caspase-3-mediated release of a lipid attraction signal. Cell. 2003; 113:717–730. [PubMed: 12809603]
20. Ravichandran KS. “Recruitment signals” from apoptotic cells: invitation to a quiet meal. Cell. 2003; 113:817–820. [PubMed: 12837239]
21. Truman LA, et al. CX3CL1/fractalkine is released from apoptotic lymphocytes to stimulate macrophage chemotaxis. Blood. 2008; 112:5026–5036. [PubMed: 18799722]
22. Gude DR, et al. Apoptosis induces expression of sphingosine kinase 1 to release sphingosine-1-phosphate as a “come-and-get-me” signal. FASEB journal: official publication of the Federation of American Societies for Experimental Biology. 2008; 22:2629–2638. [PubMed: 18362204]
23. Elliott MR, et al. Nucleotides released by apoptotic cells act as a find-me signal to promote phagocytic clearance. Nature. 2009; 461:282–286. [PubMed: 19741708]
24. Chekeni FB, et al. Pannexin 1 channels mediate 'find-me' signal release and membrane permeability during apoptosis. Nature. 2010; 467:863–867. [PubMed: 20944749]
25. Hussein MR. Apoptosis in the ovary: molecular mechanisms. Human reproduction update. 2005; 11:162–177. [PubMed: 15705959]
26. Clarke PG, Clarke S. Nineteenth century research on cell death. Experimental oncology. 2012; 34:139–145. [PubMed: 23069997]
27. Hochreiter-Hufford A, Ravichandran KS. Clearing the dead: apoptotic cell sensing, recognition, engulfment, and digestion. Cold Spring Harbor perspectives in biology. 2013; 5:a008748. [PubMed: 23284042]
28. Lauber K, Blumenthal SG, Waibel M, Wesselborg S. Clearance of apoptotic cells: getting rid of the corpses. Mol Cell. 2004; 14:277–287. [PubMed: 15125832]
29. Leventis PA, Grinstein S. The distribution and function of phosphatidylserine in cellular membranes. Annual review of biophysics. 2010; 39:407–427.

30. Borisenko GG, et al. Macrophage recognition of externalized phosphatidylserine and phagocytosis of apoptotic Jurkat cells--existence of a threshold. Arch Biochem Biophys. 2003; 413:41–52. [PubMed: 12706340]
31. Dillon SR, Constantinescu A, Schlissel MS. Annexin V binds to positively selected B cells. J Immunol. 2001; 166:58–71. [PubMed: 11123277]
32. Dillon SR, Mancini M, Rosen A, Schlissel MS. Annexin V binds to viable B cells and colocalizes with a marker of lipid rafts upon B cell receptor activation. J Immunol. 2000; 164:1322–1332. [PubMed: 10640746]
33. Frasch SC, et al. NADPH oxidase-dependent generation of lysophosphatidylserine enhances clearance of activated and dying neutrophils via G2A. J Biol Chem. 2008; 283:33736–33749. [PubMed: 18824544]
34. Frasch SC, Henson PM, Nagaosa K, Fessler MB, Borregaard N, Bratton DL. Phospholipid flip-flop and phospholipid scramblase 1 (PLSCR1) co-localize to uropod rafts in formylated Met-Leu-Phe-stimulated neutrophils. J Biol Chem. 2004; 279:17625–17633. [PubMed: 14766753]
35. Suzuki J, Umeda M, Sims PJ, Nagata S. Calcium-dependent phospholipid scrambling by TMEM16F. Nature. 2010; 468:834–838. [PubMed: 21107324]
36. Suzuki J, Denning DP, Imanishi E, Horvitz HR, Nagata S. Xk-related protein 8 and CED-8 promote phosphatidylserine exposure in apoptotic cells. Science. 2013; 341:403–406. [PubMed: 23845944]
37. Segawa K, Suzuki J, Nagata S. Constitutive exposure of phosphatidylserine on viable cells. Proc Natl Acad Sci U S A. 2011; 108:19246–19251. [PubMed: 22084121]
38. Arroyo A, et al. NADPH oxidase-dependent oxidation and externalization of phosphatidylserine during apoptosis in Me2SO-differentiated HL-60 cells. Role in phagocytic clearance. J Biol Chem. 2002; 277:49965–49975. [PubMed: 12376550]
39. Kagan VE, et al. A role for oxidative stress in apoptosis: oxidation and externalization of phosphatidylserine is required for macrophage clearance of cells undergoing Fas-mediated apoptosis. J Immunol. 2002; 169:487–499. [PubMed: 12077280]
40. Tyurin VA, et al. Oxidatively modified phosphatidylserines on the surface of apoptotic cells are essential phagocytic `eat-me' signals: cleavage and inhibition of phagocytosis by Lp-PLA2. Cell Death Differ. 2014; 21:825–835. [PubMed: 24464221]
41. Greenberg ME, Sun M, Zhang R, Febbraio M, Silverstein R, Hazen SL. Oxidized phosphatidylserine-CD36 interactions play an essential role in macrophage-dependent phagocytosis of apoptotic cells. J Exp Med. 2006; 203:2613–2625. [PubMed: 17101731]
42. Elomaa O, et al. Cloning of a novel bacteria-binding receptor structurally related to scavenger receptors and expressed in a subset of macrophages. Cell. 1995; 80:603–609. [PubMed: 7867067]
43. Wermeling F, et al. Class A scavenger receptors regulate tolerance against apoptotic cells, and autoantibodies against these receptors are predictive of systemic lupus. J Exp Med. 2007; 204:2259–2265. [PubMed: 17893199]
44. Boullier A, et al. Phosphocholine as a pattern recognition ligand for CD36. J Lipid Res. 2005; 46:969–976. [PubMed: 15722561]
45. Borisenko GG, Iverson SL, Ahlberg S, Kagan VE, Fadeel B. Milk fat globule epidermal growth factor 8 (MFG-E8) binds to oxidized phosphatidylserine: implications for macrophage clearance of apoptotic cells. Cell Death Differ. 2004; 11:943–945. [PubMed: 15031725]
46. Kimani SG, et al. Contribution of Defective PS Recognition and Efferocytosis to Chronic Inflammation and Autoimmunity. Frontiers in immunology. 2014; 5:566. [PubMed: 25426118]
47. Elward K, et al. CD46 plays a key role in tailoring innate immune recognition of apoptotic and necrotic cells. J Biol Chem. 2005; 280:36342–36354. [PubMed: 16087667]
48. Jiang H, Cooper B, Robey FA, Gewurz H. DNA binds and activates complement via residues 14–26 of the human C1q A chain. J Biol Chem. 1992; 267:25597–25601. [PubMed: 1460055]
49. Miles K, et al. A tolerogenic role for Toll-like receptor 9 is revealed by B-cell interaction with DNA complexes expressed on apoptotic cells. Proc Natl Acad Sci U S A. 2012; 109:887–892. [PubMed: 22207622]

50. Casciola-Rosen LA, Anhalt G, Rosen A. Autoantigens targeted in systemic lupus erythematosus are clustered in two populations of surface structures on apoptotic keratinocytes. J Exp Med. 1994; 179:1317–1330. [PubMed: 7511686]
51. Radic M, Marion T, Monestier M. Nucleosomes are exposed at the cell surface in apoptosis. J Immunol. 2004; 172:6692–6700. [PubMed: 15153485]
52. Paidassi H, et al. Investigations on the C1q-calreticulin-phosphatidylserine interactions yield new insights into apoptotic cell recognition. Journal of molecular biology. 2011; 408:277–290. [PubMed: 21352829]
53. Prabagar MG, et al. SIGN-R1, a C-type lectin, enhances apoptotic cell clearance through the complement deposition pathway by interacting with C1q in the spleen. Cell Death Differ. 2013; 20:535–545. [PubMed: 23238564]
54. Morelli AE, et al. Internalization of circulating apoptotic cells by splenic marginal zone dendritic cells: dependence on complement receptors and effect on cytokine production. Blood. 2003; 101:611–620. [PubMed: 12393562]
55. Ramirez-Ortiz ZG, et al. The scavenger receptor SCARF1 mediates the clearance of apoptotic cells and prevents autoimmunity. Nature immunology. 2013; 14:917–926. [PubMed: 23892722]
56. Bowness P, et al. Hereditary C1q deficiency and systemic lupus erythematosus. QJM : monthly journal of the Association of Physicians. 1994; 87:455–464. [PubMed: 7922299]
57. Walport MJ, Davies KA, Botto M. C1q and systemic lupus erythematosus. Immunobiology. 1998; 199:265–285. [PubMed: 9777411]
58. Leadbetter EA, Rifkin IR, Hohlbaum AM, Beaudette BC, Shlomchik MJ, Marshak-Rothstein A. Chromatin-IgG complexes activate B cells by dual engagement of IgM and Toll-like receptors. Nature. 2002; 416:603–607. [PubMed: 11948342]
59. Herrmann M, Voll RE, Zoller OM, Hagenhofer M, Ponner BB, Kalden JR. Impaired phagocytosis of apoptotic cell material by monocyte-derived macrophages from patients with systemic lupus erythematosus. Arthritis and rheumatism. 1998; 41:1241–1250. [PubMed: 9663482]
60. Baumann I, et al. Impaired uptake of apoptotic cells into tingible body macrophages in germinal centers of patients with systemic lupus erythematosus. Arthritis and rheumatism. 2002; 46:191–201. [PubMed: 11817590]
61. Arbuckle MR, et al. Development of autoantibodies before the clinical onset of systemic lupus erythematosus. N Engl J Med. 2003; 349:1526–1533. [PubMed: 14561795]
62. Nydegger UE, Lambert PH, Gerber H, Miescher PA. Circulating immune complexes in the serum in systemic lupus erythematosus and in carriers of hepatitis B antigen. Quantitation by binding to radiolabeled C1q. J Clin Invest. 1974; 54:297–309. [PubMed: 4847246]
63. Cano PO, Jerry LM, Sladowski JP, Osterland CK. Circulating immune complexes in systemic lupus erythematosus. Clinical and experimental immunology. 1977; 29:197–204. [PubMed: 302768]
64. Christensen SR, Kashgarian M, Alexopoulou L, Flavell RA, Akira S, Shlomchik MJ. Toll-like receptor 9 controls anti-DNA autoantibody production in murine lupus. J Exp Med. 2005; 202:321–331. [PubMed: 16027240]
65. Christensen SR, Shupe J, Nickerson K, Kashgarian M, Flavell RA, Shlomchik MJ. Toll-like receptor 7 and TLR9 dictate autoantibody specificity and have opposing inflammatory and regulatory roles in a murine model of lupus. Immunity. 2006; 25:417–428. [PubMed: 16973389]
66. Pisitkun P, Deane JA, Difilippantonio MJ, Tarasenko T, Satterthwaite AB, Bolland S. Autoreactive B cell responses to RNA-related antigens due to TLR7 gene duplication. Science. 2006; 312:1669–1672. [PubMed: 16709748]
67. Deane JA, et al. Control of toll-like receptor 7 expression is essential to restrict autoimmunity and dendritic cell proliferation. Immunity. 2007; 27:801–810. [PubMed: 17997333]
68. McGaha TL, Madaio MP. Lupus Nephritis: Animal Modeling of a Complex Disease Syndrome Pathology. Drug discovery today Disease models. 2014; 11:13–18. [PubMed: 25722732]
69. Nickerson KM, Cullen JL, Kashgarian M, Shlomchik MJ. Exacerbated autoimmunity in the absence of TLR9 in MRL.Fas(lpr) mice depends on Ifnar1. J Immunol. 2013; 190:3889–3894. [PubMed: 23467932]

70. Sims GP, Rowe DC, Rietdijk ST, Herbst R, Coyle AJ. HMGB1 and RAGE in inflammation and cancer. Annual review of immunology. 2010; 28:367–388.
71. Avalos AM, et al. RAGE-independent autoreactive B cell activation in response to chromatin and HMGB1/DNA immune complexes. Autoimmunity. 2010; 43:103–110. [PubMed: 20014975]
72. Kazama H, Ricci JE, Herndon JM, Hoppe G, Green DR, Ferguson TA. Induction of immunological tolerance by apoptotic cells requires caspase-dependent oxidation of high-mobility group box-1 protein. Immunity. 2008; 29:21–32. [PubMed: 18631454]
73. Huang L, et al. Engineering DNA nanoparticles as immunomodulatory reagents that activate regulatory T cells. J Immunol. 2012; 188:4913–4920. [PubMed: 22516958]
74. Holm CK, Paludan SR, Fitzgerald KA. DNA recognition in immunity and disease. Current opinion in immunology. 2013; 25:13–18. [PubMed: 23313533]
75. Huang L, et al. Cutting edge: DNA sensing via the STING adaptor in myeloid dendritic cells induces potent tolerogenic responses. J Immunol. 2013; 191:3509–3513. [PubMed: 23986532]
76. Ravishankar B, et al. Tolerance to apoptotic cells is regulated by indoleamine 2,3-dioxygenase. Proc Natl Acad Sci U S A. 2012; 109:3909–3914. [PubMed: 22355111]
77. Sharma S, et al. Suppression of systemic autoimmunity by the innate immune adaptor STING. Proc Natl Acad Sci U S A. 2015; 112:E710–717. [PubMed: 25646421]
78. Stetson DB, Ko JS, Heidmann T, Medzhitov R. Trex1 prevents cell-intrinsic initiation of autoimmunity. Cell. 2008; 134:587–598. [PubMed: 18724932]
79. Gall A, et al. Autoimmunity initiates in nonhematopoietic cells and progresses via lymphocytes in an interferon-dependent autoimmune disease. Immunity. 2012; 36:120–131. [PubMed: 22284419]
80. White MJ, et al. Apoptotic caspases suppress mtDNA-induced STING-mediated type I IFN production. Cell. 2014; 159:1549–1562. [PubMed: 25525874]
81. Rongvaux A, et al. Apoptotic caspases prevent the induction of type I interferons by mitochondrial DNA. Cell. 2014; 159:1563–1577. [PubMed: 25525875]
82. Woo SR, et al. STING-dependent cytosolic DNA sensing mediates innate immune recognition of immunogenic tumors. Immunity. 2014; 41:830–842. [PubMed: 25517615]
83. Deng L, et al. STING-Dependent Cytosolic DNA Sensing Promotes Radiation-Induced Type I Interferon-Dependent Antitumor Immunity in Immunogenic Tumors. Immunity. 2014; 41:843–852. [PubMed: 25517616]
84. Weaver BA, Cleveland DW. Does aneuploidy cause cancer? Current opinion in cell biology. 2006; 18:658–667. [PubMed: 17046232]
85. Kim JJ, Tannock IF. Repopulation of cancer cells during therapy: an important cause of treatment failure. Nature reviews Cancer. 2005; 5:516–525. [PubMed: 15965493]
86. Kawane K, et al. Chronic polyarthritis caused by mammalian DNA that escapes from degradation in macrophages. Nature. 2006; 443:998–1002. [PubMed: 17066036]
87. Ahn J, Gutman D, Saijo S, Barber GN. STING manifests self DNA-dependent inflammatory disease. Proc Natl Acad Sci U S A. 2012; 109:19386–19391. [PubMed: 23132945]
88. Amlot PL, Hayes AE. Impaired human antibody response to the thymus-independent antigen, DNP-Ficoll, after splenectomy. Implications for post-splenectomy infections. Lancet. 1985; 1:1008–1011. [PubMed: 2859463]
89. Kruetzmann S, et al. Human immunoglobulin M memory B cells controlling Streptococcus pneumoniae infections are generated in the spleen. J Exp Med. 2003; 197:939–945. [PubMed: 12682112]
90. Castagnola E, Fioredda F. Prevention of life-threatening infections due to encapsulated bacteria in children with hyposplenia or asplenia: a brief review of current recommendations for practical purposes. European journal of haematology. 2003; 71:319–326. [PubMed: 14667194]
91. Mebius RE, Kraal G. Structure and function of the spleen. Nature reviews Immunology. 2005; 5:606–616.
92. Cerutti A, Cols M, Puga I. Marginal zone B cells: virtues of innate-like antibody-producing lymphocytes. Nature reviews Immunology. 2013; 13:118–132.
93. Savage HP, Baumgarth N. Characteristics of natural antibody-secreting cells. Annals of the New York Academy of Sciences. 2015

94. Kusumi S, Koga D, Kanda T, Ushiki T. Three-dimensional reconstruction of serial sections for analysis of the microvasculature of the white pulp and the marginal zone in the human spleen. Biomedical research. 2015; 36:195–203. [PubMed: 26106049]

95. Yeramilli VA, Knight KL. Development of CD27+ marginal zone B cells requires GALT. European journal of immunology. 2013; 43:1484–1488. [PubMed: 23468368]

96. Chung JB, Silverman M, Monroe JG. Transitional B cells: step by step towards immune competence. Trends Immunol. 2003; 24:343–349. [PubMed: 12810111]

97. Meffre E, Wardemann H. B-cell tolerance checkpoints in health and autoimmunity. Current opinion in immunology. 2008; 20:632–638. [PubMed: 18848883]

98. Tanigaki K, et al. Notch-RBP-J signaling is involved in cell fate determination of marginal zone B cells. Nature immunology. 2002; 3:443–450. [PubMed: 11967543]

99. Hampel F, et al. CD19-independent instruction of murine marginal zone B-cell development by constitutive Notch2 signaling. Blood. 2011; 118:6321–6331. [PubMed: 21795747]

100. Silverman GJ. Protective natural autoantibodies to apoptotic cells: evidence of convergent selection of recurrent innate-like clones. Annals of the New York Academy of Sciences. 2015

101. Dunn-Walters DK, Isaacson PG, Spencer J. Analysis of mutations in immunoglobulin heavy chain variable region genes of microdissected marginal zone (MGZ) B cells suggests that the MGZ of human spleen is a reservoir of memory B cells. J Exp Med. 1995; 182:559–566. [PubMed: 7629512]

102. Descatoire M, et al. Identification of a human splenic marginal zone B cell precursor with NOTCH2-dependent differentiation properties. J Exp Med. 2014; 211:987–1000. [PubMed: 24733829]

103. Weller S, et al. CD40-CD40L independent Ig gene hypermutation suggests a second B cell diversification pathway in humans. Proc Natl Acad Sci U S A. 2001; 98:1166–1170. [PubMed: 11158612]

104. Tarlinton D. Sheepish B cells: evidence for antigen-independent antibody diversification in humans and mice. J Exp Med. 2008; 205:1251–1254. [PubMed: 18519651]

105. Bemark M. Translating transitions - how to decipher peripheral human B cell development. Journal of biomedical research. 2015; 29:264–284. [PubMed: 26243514]

106. Arnon TI, Horton RM, Grigorova IL, Cyster JG. Visualization of splenic marginal zone B-cell shuttling and follicular B-cell egress. Nature. 2013; 493:684–688. [PubMed: 23263181]

107. Guinamard R, Okigaki M, Schlessinger J, Ravetch JV. Absence of marginal zone B cells in Pyk-2-deficient mice defines their role in the humoral response. Nature immunology. 2000; 1:31–36. [PubMed: 10881171]

108. Hjelm F, Karlsson MC, Heyman B. A novel B cell-mediated transport of IgE-immune complexes to the follicle of the spleen. J Immunol. 2008; 180:6604–6610. [PubMed: 18453579]

109. Won WJ, Bachmann MF, Kearney JF. CD36 is differentially expressed on B cell subsets during development and in responses to antigen. J Immunol. 2008; 180:230–237. [PubMed: 18097024]

110. Barr TA, Brown S, Ryan G, Zhao J, Gray D. TLR-mediated stimulation of APC: Distinct cytokine responses of B cells and dendritic cells. European journal of immunology. 2007; 37:3040–3053. [PubMed: 17918201]

111. Won WJ, Kearney JF. CD9 is a unique marker for marginal zone B cells, B1 cells, and plasma cells in mice. J Immunol. 2002; 168:5605–5611. [PubMed: 12023357]

112. den Haan JM, Mebius RE, Kraal G. Stromal cells of the mouse spleen. Frontiers in immunology. 2012; 3:201. [PubMed: 22807924]

113. Katakai T, et al. Organizer-like reticular stromal cell layer common to adult secondary lymphoid organs. J Immunol. 2008; 181:6189–6200. [PubMed: 18941209]

114. Cremasco V, et al. B cell homeostasis and follicle confines are governed by fibroblastic reticular cells. Nature immunology. 2014; 15:973–981. [PubMed: 25151489]

115. Heesters BA, Myers RC, Carroll MC. Follicular dendritic cells: dynamic antigen libraries. Nature reviews Immunology. 2014; 14:495–504.

116. Swirski FK, et al. Identification of splenic reservoir monocytes and their deployment to inflammatory sites. Science. 2009; 325:612–616. [PubMed: 19644120]

117. Ribechini E, Greifenberg V, Sandwick S, Lutz MB. Subsets, expansion and activation of myeloid-derived suppressor cells. Medical microbiology and immunology. 2010; 199:273–281. [PubMed: 20376485]
118. Puga I, et al. B cell-helper neutrophils stimulate the diversification and production of immunoglobulin in the marginal zone of the spleen. Nature immunology. 2012; 13:170–180. [PubMed: 22197976]
119. Magri G, et al. Innate lymphoid cells integrate stromal and immunological signals to enhance antibody production by splenic marginal zone B cells. Nature immunology. 2014; 15:354–364. [PubMed: 24562309]
120. Sonnenberg GF, Artis D. Innate lymphoid cells in the initiation, regulation and resolution of inflammation. Nature medicine. 2015; 21:698–708.
121. den Haan JM, Kraal G. Innate immune functions of macrophage subpopulations in the spleen. Journal of innate immunity. 2012; 4:437–445. [PubMed: 22327291]
122. Rahman ZS, Shao WH, Khan TN, Zhen Y, Cohen PL. Impaired apoptotic cell clearance in the germinal center by Mer-deficient tingible body macrophages leads to enhanced antibody-forming cell and germinal center responses. J Immunol. 2010; 185:5859–5868. [PubMed: 20952679]
123. Kranich J, et al. Follicular dendritic cells control engulfment of apoptotic bodies by secreting Mfge8. J Exp Med. 2008; 205:1293–1302. [PubMed: 18490487]
124. Knutson M, Wessling-Resnick M. Iron metabolism in the reticuloendothelial system. Critical reviews in biochemistry and molecular biology. 2003; 38:61–88. [PubMed: 12641343]
125. Roozendaal R, et al. Conduits mediate transport of low-molecular-weight antigen to lymph node follicles. Immunity. 2009; 30:264–276. [PubMed: 19185517]
126. Grasset EK, et al. Sterile inflammation in the spleen during atherosclerosis provides oxidation-specific epitopes that induce a protective B-cell response. Proc Natl Acad Sci U S A. 2015; 112:E2030–2038. [PubMed: 25848033]
127. Kang YS, et al. The C-type lectin SIGN-R1 mediates uptake of the capsular polysaccharide of Streptococcus pneumoniae in the marginal zone of mouse spleen. Proc Natl Acad Sci U S A. 2004; 101:215–220. [PubMed: 14694198]
128. You Y, et al. Marginal zone B cells regulate antigen capture by marginal zone macrophages. J Immunol. 2011; 186:2172–2181. [PubMed: 21257969]
129. Karlsson MC, Guinamard R, Bolland S, Sankala M, Steinman RM, Ravetch JV. Macrophages control the retention and trafficking of B lymphocytes in the splenic marginal zone. J Exp Med. 2003; 198:333–340. [PubMed: 12874264]
130. Kang YS, et al. A dominant complement fixation pathway for pneumococcal polysaccharides initiated by SIGN-R1 interacting with C1q. Cell. 2006; 125:47–58. [PubMed: 16615889]
131. Vomhof-DeKrey EE, et al. Cognate interaction with iNKT cells expands IL-10-producing B regulatory cells. Proceedings of the National Academy of Sciences of the United States of America. 2015; 112:12474–12479. [PubMed: 26392556]
132. King IL, et al. Invariant natural killer T cells direct B cell responses to cognate lipid antigen in an IL-21-dependent manner. Nature immunology. 2012; 13:44–50. [PubMed: 22120118]
133. Vomhof-DeKrey EE, et al. Cognate interaction with iNKT cells expands IL-10-producing B regulatory cells. Proc Natl Acad Sci U S A. 2015
134. Maseda D, et al. Regulatory B10 cells differentiate into antibody-secreting cells after transient IL-10 production in vivo. J Immunol. 2012; 188:1036–1048. [PubMed: 22198952]
135. Enoksson SL, et al. The inflammatory cytokine IL-18 induces self-reactive innate antibody responses regulated by natural killer T cells. Proc Natl Acad Sci U S A. 2011; 108:E1399–1407. [PubMed: 22135456]
136. Wermeling F, Lind SM, Jordo ED, Cardell SL, Karlsson MC. Invariant NKT cells limit activation of autoreactive CD1d-positive B cells. J Exp Med. 2010; 207:943–952. [PubMed: 20439539]
137. Kain L, et al. Endogenous ligands of natural killer T cells are alpha-linked glycosylceramides. Molecular immunology. 2015
138. Li Y, et al. CD4+ natural killer T cells potently augment aortic root atherosclerosis by perforin- and granzyme B-dependent cytotoxicity. Circulation research. 2015; 116:245–254. [PubMed: 25398236]

139. Nowak M, Kopp F, Roelofs-Haarhuis K, Wu X, Gleichmann E. Oral nickel tolerance: Fas ligand-expressing invariant NK T cells promote tolerance induction by eliciting apoptotic death of antigen-carrying, effete B cells. J Immunol. 2006; 176:4581–4589. [PubMed: 16585548]
140. Wingender G, Krebs P, Beutler B, Kronenberg M. Antigen-specific cytotoxicity by invariant NKT cells in vivo is CD95/CD178-dependent and is correlated with antigenic potency. J Immunol. 2010; 185:2721–2729. [PubMed: 20660713]
141. Sonoda KH, Exley M, Snapper S, Balk SP, Stein-Streilein J. CD1-reactive natural killer T cells are required for development of systemic tolerance through an immune-privileged site. J Exp Med. 1999; 190:1215–1226. [PubMed: 10544194]
142. Kraal G, Mebius R. New insights into the cell biology of the marginal zone of the spleen. International review of cytology. 2006; 250:175–215. [PubMed: 16861066]
143. Steiniger BS. Human spleen microanatomy: why mice do not suffice. Immunology. 2015; 145:334–346. [PubMed: 25827019]
144. Steiniger B, Trabandt M, Barth PJ. The follicular dendritic cell network in secondary follicles of human palatine tonsils and spleens. Histochemistry and cell biology. 2011; 135:327–336. [PubMed: 21394653]
145. Steiniger B, Barth P, Hellinger A. The perifollicular and marginal zones of the human splenic white pulp : do fibroblasts guide lymphocyte immigration? The American journal of pathology. 2001; 159:501–512. [PubMed: 11485909]
146. Steiniger B, Barth P, Herbst B, Hartnell A, Crocker PR. The species-specific structure of microanatomical compartments in the human spleen: strongly sialoadhesin-positive macrophages occur in the perifollicular zone, but not in the marginal zone. Immunology. 1997; 92:307–316. [PubMed: 9415041]
147. O'Neill AS, van den Berg TK, Mullen GE. Sialoadhesin - a macrophage-restricted marker of immunoregulation and inflammation. Immunology. 2013; 138:198–207. [PubMed: 23181380]
148. Steiniger B, Timphus EM, Barth PJ. The splenic marginal zone in humans and rodents: an enigmatic compartment and its inhabitants. Histochemistry and cell biology. 2006; 126:641–648. [PubMed: 16816939]
149. Steiniger B, Bette M, Schwarzbach H. The open microcirculation in human spleens: a three-dimensional approach. The journal of histochemistry and cytochemistry : official journal of the Histochemistry Society. 2011; 59:639–648. [PubMed: 21525186]
150. Schmitt S, Johnson TS, Karakhanova S, Naher H, Mahnke K, Enk AH. Extracorporeal photophoresis augments function of CD4+CD25+FoxP3+ regulatory T cells by triggering adenosine production. Transplantation. 2009; 88:411–416. [PubMed: 19667946]
151. Ravishankar B, et al. Marginal zone CD169+ macrophages coordinate apoptotic cell-driven cellular recruitment and tolerance. Proc Natl Acad Sci U S A. 2014; 111:4215–4220. [PubMed: 24591636]
152. Benden C, et al. Extracorporeal photopheresis after lung transplantation: a 10-year single-center experience. Transplantation. 2008; 86:1625–1627. [PubMed: 19077900]
153. Hivelin M, Siemionow M, Grimbert P, Lantieri L. Extracorporeal photopheresis: from solid organs to face transplantation. Transplant immunology. 2009; 21:117–128. [PubMed: 19409991]
154. Kumlien G, Genberg H, Shanwell A, Tyden G. Photopheresis for the treatment of refractory renal graft rejection. Transplantation. 2005; 79:123–125. [PubMed: 15714180]
155. Lutterotti A, et al. Antigen-specific tolerance by autologous myelin peptide-coupled cells: a phase 1 trial in multiple sclerosis. Science translational medicine. 2013; 5:188ra175.
156. Kleinclauss F, et al. Intravenous apoptotic spleen cell infusion induces a TGF-beta-dependent regulatory T-cell expansion. Cell Death Differ. 2006; 13:41–52. [PubMed: 15962005]
157. Ravishankar B, et al. The amino acid sensor GCN2 inhibits inflammatory responses to apoptotic cells promoting tolerance and suppressing systemic autoimmunity. Proc Natl Acad Sci U S A. 2015; 112:10774–10779. [PubMed: 26261340]
158. McGaha TL, Chen Y, Ravishankar B, van Rooijen N, Karlsson MC. Marginal zone macrophages suppress innate and adaptive immunity to apoptotic cells in the spleen. Blood. 2011; 117:5403–5412. [PubMed: 21444914]

159. Miyake Y, Asano K, Kaise H, Uemura M, Nakayama M, Tanaka M. Critical role of macrophages in the marginal zone in the suppression of immune responses to apoptotic cell-associated antigens. J Clin Invest. 2007; 117:2268–2278. [PubMed: 17657313]

160. Qiu CH, Miyake Y, Kaise H, Kitamura H, Ohara O, Tanaka M. Novel subset of CD8{alpha}+ dendritic cells localized in the marginal zone is responsible for tolerance to cell-associated antigens. J Immunol. 2009; 182:4127–4136. [PubMed: 19299710]

161. Dai W, Gupta SL. Regulation of indoleamine 2,3-dioxygenase gene expression in human fibroblasts by interferon-gamma. Upstream control region discriminates between interferon-gamma and interferon-alpha. J Biol Chem. 1990; 265:19871–19877. [PubMed: 2174056]

162. Hassanain HH, Chon SY, Gupta SL. Differential regulation of human indoleamine 2,3-dioxygenase gene expression by interferons-gamma and -alpha. Analysis of the regulatory region of the gene and identification of an interferon-gamma-inducible DNA-binding factor. J Biol Chem. 1993; 268:5077–5084. [PubMed: 8444884]

163. McGaha TL, et al. Amino acid catabolism: a pivotal regulator of innate and adaptive immunity. Immunological reviews. 2012; 249:135–157. [PubMed: 22889220]

164. Dong J, Qiu H, Garcia-Barrio M, Anderson J, Hinnebusch AG. Uncharged tRNA activates GCN2 by displacing the protein kinase moiety from a bipartite tRNA-binding domain. Mol Cell. 2000; 6:269–279. [PubMed: 10983975]

165. Baird TD, Wek RC. Eukaryotic initiation factor 2 phosphorylation and translational control in metabolism. Advances in nutrition. 2012; 3:307–321. [PubMed: 22585904]

166. Kim S, Elkon KB, Ma X. Transcriptional suppression of interleukin-12 gene expression following phagocytosis of apoptotic cells. Immunity. 2004; 21:643–653. [PubMed: 15539151]

167. Godiska R, et al. Human macrophage-derived chemokine (MDC), a novel chemoattractant for monocytes, monocyte-derived dendritic cells, and natural killer cells. J Exp Med. 1997; 185:1595–1604. [PubMed: 9151897]

168. Nishikawa H, Sakaguchi S. Regulatory T cells in tumor immunity. Int J Cancer. 2010; 127:759–767. [PubMed: 20518016]

169. Bayry J, et al. In silico identified CCR4 antagonists target regulatory T cells and exert adjuvant activity in vaccination. Proc Natl Acad Sci U S A. 2008; 105:10221–10226. [PubMed: 18621704]

170. Gobert M, et al. Regulatory T cells recruited through CCL22/CCR4 are selectively activated in lymphoid infiltrates surrounding primary breast tumors and lead to an adverse clinical outcome. Cancer Res. 2009; 69:2000–2009. [PubMed: 19244125]

171. Pere H, et al. A CCR4 antagonist combined with vaccines induces antigen-specific CD8+ T cells and tumor immunity against self antigens. Blood. 2011; 118:4853–4862. [PubMed: 21908423]

172. Lee I, Wang L, Wells AD, Dorf ME, Ozkaynak E, Hancock WW. Recruitment of Foxp3+ T regulatory cells mediating allograft tolerance depends on the CCR4 chemokine receptor. J Exp Med. 2005; 201:1037–1044. [PubMed: 15809349]

173. Yu K, Chen Z, Khatri I, Gorczynski RM. CCR4 dependent migration of Foxp3+ Treg cells to skin grafts and draining lymph nodes is implicated in enhanced graft survival in CD200tg recipients. Immunol Lett. 2011; 141:116–122. [PubMed: 21945175]

174. Montane J, et al. Prevention of murine autoimmune diabetes by CCL22-mediated Treg recruitment to the pancreatic islets. J Clin Invest. 2011; 121:3024–3028. [PubMed: 21737880]

175. Poppensieker K, et al. CC chemokine receptor 4 is required for experimental autoimmune encephalomyelitis by regulating GM-CSF and IL-23 production in dendritic cells. Proc Natl Acad Sci U S A. 2012; 109:3897–3902. [PubMed: 22355103]

176. Bajwa A, et al. Sphingosine 1-Phosphate Receptor 3-Deficient Dendritic Cells Modulate Splenic Responses to Ischemia-Reperfusion Injury. Journal of the American Society of Nephrology : JASN. 2015

177. Reizis B, Bunin A, Ghosh HS, Lewis KL, Sisirak V. Plasmacytoid dendritic cells: recent progress and open questions. Annual review of immunology. 2011; 29:163–183.

178. Ohnmacht C, et al. Constitutive ablation of dendritic cells breaks self-tolerance of CD4 T cells and results in spontaneous fatal autoimmunity. J Exp Med. 2009; 206:549–559. [PubMed: 19237601]

179. Chen M, et al. Dendritic cell apoptosis in the maintenance of immune tolerance. Science. 2006; 311:1160–1164. [PubMed: 16497935]

180. Sharma MD, et al. An inherently bifunctional subset of Foxp3+ T helper cells is controlled by the transcription factor eos. Immunity. 2013; 38:998–1012. [PubMed: 23684987]

181. Rosenblum MD, Gratz IK, Paw JS, Lee K, Marshak-Rothstein A, Abbas AK. Response to self antigen imprints regulatory memory in tissues. Nature. 2011; 480:538–542. [PubMed: 22121024]

182. Desch AN, et al. CD103+ pulmonary dendritic cells preferentially acquire and present apoptotic cell-associated antigen. J Exp Med. 2011; 208:1789–1797. [PubMed: 21859845]

183. Iyoda T, et al. The CD8+ dendritic cell subset selectively endocytoses dying cells in culture and in vivo. J Exp Med. 2002; 195:1289–1302. [PubMed: 12021309]

184. Liu K, Iyoda T, Saternus M, Kimura Y, Inaba K, Steinman RM. Immune tolerance after delivery of dying cells to dendritic cells in situ. J Exp Med. 2002; 196:1091–1097. [PubMed: 12391020]

185. Qiu CH, Miyake Y, Kaise H, Kitamura H, Ohara O, Tanaka M. Novel subset of CD8{alpha}+ dendritic cells localized in the marginal zone is responsible for tolerance to cell-associated antigens. J Immunol. 2009; 182:4127–4136. [PubMed: 19299710]

186. den Haan JM, Lehar SM, Bevan MJ. CD8(+) but not CD8(−) dendritic cells cross-prime cytotoxic T cells in vivo. J Exp Med. 2000; 192:1685–1696. [PubMed: 11120766]

187. Pooley JL, Heath WR, Shortman K. Cutting edge: intravenous soluble antigen is presented to CD4 T cells by CD8− dendritic cells, but cross-presented to CD8 T cells by CD8+ dendritic cells. J Immunol. 2001; 166:5327–5330. [PubMed: 11313367]

188. Yamazaki S, et al. CD8+ CD205+ splenic dendritic cells are specialized to induce Foxp3+ regulatory T cells. J Immunol. 2008; 181:6923–6933. [PubMed: 18981112]

189. Inaba K, et al. Efficient presentation of phagocytosed cellular fragments on the major histocompatibility complex class II products of dendritic cells. J Exp Med. 1998; 188:2163–2173. [PubMed: 9841929]

190. Schiller M, et al. Induction of type I IFN is a physiological immune reaction to apoptotic cell-derived membrane microparticles. J Immunol. 2012; 189:1747–1756. [PubMed: 22786771]

191. Bonnefoy F, et al. Plasmacytoid dendritic cells play a major role in apoptotic leukocyte-induced immune modulation. J Immunol. 2011; 186:5696–5705. [PubMed: 21460208]

192. Ito T, et al. Plasmacytoid dendritic cells prime IL-10-producing T regulatory cells by inducible costimulator ligand. J Exp Med. 2007; 204:105–115. [PubMed: 17200410]

193. Manches O, et al. HIV-activated human plasmacytoid DCs induce Tregs through an indoleamine 2,3-dioxygenase-dependent mechanism. J Clin Invest. 2008; 118:3431–3439. [PubMed: 18776940]

194. Sharma MD, et al. Plasmacytoid dendritic cells from mouse tumor-draining lymph nodes directly activate mature Tregs via indoleamine 2,3-dioxygenase. J Clin Invest. 2007; 117:2570–2582. [PubMed: 17710230]

195. Hooijkaas H, Benner R, Pleasants JR, Wostmann BS. ISOTYPES AND SPECIFICITIES OF IMMUNOGLOBULINS PRODUCED BY GERM-FREE MICE FED CHEMICALLY DEFINED ULTRAFILTERED ANTIGEN-FREE DIET. European journal of immunology. 1984; 14:1127–1130. [PubMed: 6083871]

196. Chou MY, et al. Oxidation-specific epitopes are dominant targets of innate natural antibodies in mice and humans. J Clin Invest. 2009; 119:1335–1349. [PubMed: 19363291]

197. Lalor PA, Herzenberg LA, Adams S, Stall AM. Feedback regulation of murine Ly-1 B cell development. European journal of immunology. 1989; 19:507–513. [PubMed: 2785046]

198. Vas J, Gronwall C, Silverman GJ. Fundamental roles of the innate-like repertoire of natural antibodies in immune homeostasis. Frontiers in immunology. 2013; 4:4. [PubMed: 23386848]

199. Feeney AJ. Predominance of the prototypic T15 anti-phosphorylcholine junctional sequence in neonatal pre-B cells. J Immunol. 1991; 147:4343–4350. [PubMed: 1753104]

200. Briles DE, Forman C, Hudak S, Claflin JL. Anti-phosphorylcholine antibodies of the T15 idiotype are optimally protective against Streptococcus pneumoniae. J Exp Med. 1982; 156:1177–1185. [PubMed: 7153709]

201. Sigal NH, Gearhart PJ, Klinman NR. The frequency of phosphorylcholine-specific B cells in conventional and germfree BALB/C mice. J Immunol. 1975; 114:1354–1358. [PubMed: 1078833]

202. Claflin JL, Cubberley M. Clonal nature of the immune response to phosphocholine. VII. Evidence throughout inbred mice for molecular similarities among antibodies bearing the T15 idiotype. J Immunol. 1980; 125:551–558. [PubMed: 7391569]

203. Shaw PX, et al. Natural antibodies with the T15 idiotype may act in atherosclerosis, apoptotic clearance, and protective immunity. J Clin Invest. 2000; 105:1731–1740. [PubMed: 10862788]

204. Gronwall C, et al. MAPK phosphatase-1 is required for regulatory natural autoantibody-mediated inhibition of TLR responses. Proc Natl Acad Sci U S A. 2012; 109:19745–19750. [PubMed: 23139409]

205. Chen Y, Park YB, Patel E, Silverman GJ. IgM antibodies to apoptosis-associated determinants recruit C1q and enhance dendritic cell phagocytosis of apoptotic cells. J Immunol. 2009; 182:6031–6043. [PubMed: 19414754]

206. Merle NS, Noe R, Halbwachs-Mecarelli L, Fremeaux-Bacchi V, Roumenina LT. Complement System Part II: Role in Immunity. Frontiers in immunology. 2015; 6:257. [PubMed: 26074922]

207. Roozendaal R, Carroll MC. Complement receptors CD21 and CD35 in humoral immunity. Immunol Rev. 2007; 219:157–166. [PubMed: 17850488]

208. Muller-Eberhard HJ. Molecular organization and function of the complement system. Annu Rev Biochem. 1988; 57:321–347. [PubMed: 3052276]

209. Kojouharova M, Reid K, Gadjeva M. New insights into the molecular mechanisms of classical complement activation. Molecular immunology. 2010; 47:2154–2160. [PubMed: 20542571]

210. Cooper NR, Nemerow GR, Mayes JT. Methods to detect and quantitate complement activation. Springer Semin Immunopathol. 1983; 6:195–212. [PubMed: 6195745]

211. Borsos T, Rapp HJ. Complement fixation on cell surfaces by 19S and 7S antibodies. Science. 1965; 150:505–506. [PubMed: 5319759]

212. Fishelson Z, Attali G, Mevorach D. Complement and apoptosis. Molecular immunology. 2001; 38:207–219. [PubMed: 11532282]

213. Benoit ME, Clarke EV, Morgado P, Fraser DA, Tenner AJ. Complement protein C1q directs macrophage polarization and limits inflammasome activity during the uptake of apoptotic cells. J Immunol. 2012; 188:5682–5693. [PubMed: 22523386]

214. Clarke EV, Weist BM, Walsh CM, Tenner AJ. Complement protein C1q bound to apoptotic cells suppresses human macrophage and dendritic cell-mediated Th17 and Th1 T cell subset proliferation. Journal of leukocyte biology. 2015; 97:147–160. [PubMed: 25381385]

215. Amarilyo G, et al. iC3b-opsonized apoptotic cells mediate a distinct anti-inflammatory response and transcriptional NF-kappaB-dependent blockade. European journal of immunology. 2010; 40:699–709. [PubMed: 20039295]

216. Dempsey PW, Allison ME, Akkaraju S, Goodnow CC, Fearon DT. C3d of complement as a molecular adjuvant: bridging innate and acquired immunity. Science. 1996; 271:348–350. [PubMed: 8553069]

217. Cinamon G, Zachariah MA, Lam OM, Foss FW Jr, Cyster JG. Follicular shuttling of marginal zone B cells facilitates antigen transport. Nature immunology. 2008; 9:54–62. [PubMed: 18037889]

218. Heesters BA, Das A, Chatterjee P, Carroll MC. Do follicular dendritic cells regulate lupus-specific B cells? Molecular immunology. 2014; 62:283–288. [PubMed: 24636642]

219. Jordo ED, Wermeling F, Chen Y, Karlsson MC. Scavenger receptors as regulators of natural antibody responses and B cell activation in autoimmunity. Molecular immunology. 2011; 48:1307–1318. [PubMed: 21333356]

220. Lewis MJ, Malik TH, Ehrenstein MR, Boyle JJ, Botto M, Haskard DO. Immunoglobulin M is required for protection against atherosclerosis in low-density lipoprotein receptor-deficient mice. Circulation. 2012; 120:417–426. [PubMed: 19620499]

221. Rosen A, Casciola-Rosen L. Autoantigens as substrates for apoptotic proteases: implications for the pathogenesis of systemic autoimmune disease. Cell death and differentiation. 1999; 6:6–12. [PubMed: 10200542]

222. Birjandi SZ, Ippolito JA, Ramadorai AK, Witte PL. Alterations in marginal zone macrophages and marginal zone B cells in old mice. J Immunol. 2011; 186:3441–3451. [PubMed: 21307289]

223. Kraal G, Schornagel K, Streeter PR, Holzmann B, Butcher EC. Expression of the mucosal vascular addressin, MAdCAM-1, on sinus-lining cells in the spleen. Am J Pathol. 1995; 147:763–771. [PubMed: 7677187]

224. Tanaka H, Hataba Y, Saito S, Fukushima O, Miyasaka M. Phenotypic characteristics and significance of reticular meshwork surrounding splenic white pulp of mice. J Electron Microsc (Tokyo). 1996; 45:407–416. [PubMed: 9041702]

225. Girkontaite I, et al. The sphingosine-1-phosphate (S1P) lysophospholipid receptor S1P3 regulates MAdCAM-1+ endothelial cells in splenic marginal sinus organization. J Exp Med. 2004; 200:1491–1501. [PubMed: 15583019]

226. Li H, et al. Cutting Edge: defective follicular exclusion of apoptotic antigens due to marginal zone macrophage defects in autoimmune BXD2 mice. J Immunol. 2013; 190:4465–4469. [PubMed: 23543760]

227. Li H, et al. Interferon-induced mechanosensing defects impede apoptotic cell clearance in lupus. J Clin Invest. 2015; 125:2877–2890. [PubMed: 26098211]

228. Binder CJ. Naturally occurring IgM antibodies to oxidation-specific epitopes. Advances in experimental medicine and biology. 2012; 750:2–13. [PubMed: 22903662]

229. Wang C, et al. Natural antibodies of newborns recognize oxidative stress-related malondialdehyde acetaldehyde adducts on apoptotic cells and atherosclerotic plaques. International immunology. 2013; 25:575–587. [PubMed: 23900424]

230. Kyaw T, et al. Conventional B2 B cell depletion ameliorates whereas its adoptive transfer aggravates atherosclerosis. J Immunol. 2010; 185:4410–4419. [PubMed: 20817865]

231. Ait-Oufella H, et al. B cell depletion reduces the development of atherosclerosis in mice. J Exp Med. 2010; 207:1579–1587. [PubMed: 20603314]

232. Caligiuri G, Nicoletti A, Poirier B, Hansson GK. Protective immunity against atherosclerosis carried by B cells of hypercholesterolemic mice. J Clin Invest. 2002; 109:745–753. [PubMed: 11901183]

233. Robinette CD, Fraumeni JF Jr. Splenectomy and subsequent mortality in veterans of the 1939–45 war. Lancet. 1977; 2:127–129. [PubMed: 69206]

234. Aviram M, Brook JG, Tatarsky I, Levy Y, Carter A. Increased low-density lipoprotein levels after splenectomy: a role for the spleen in cholesterol metabolism in myeloproliferative disorders. The American journal of the medical sciences. 1986; 291:25–28. [PubMed: 3942157]