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Autoimmune Pathogenesis and Autoimmune Hemolytic Anemia

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Autoimmune hemolytic anemia (AIHA) is an autoimmune disorder in which autoantibodies are directed against an individual's own red blood cells (RBCs), leading to enhanced clearance through Fc receptor (FcR)-mediated phagocytosis. Although there is a large literature relating to clinical aspects of AIHA, relatively little work addresses how IgG autoantibodies are actually produced against RBC autoantigens. This review will first discuss the current understanding of autoimmunity in general and then focus on the knowledge of the immunopathogenic mechanisms responsible for autoantibody production in AIHA. Both human and animal studies will be discussed. Understanding theses mechanism is vital for developing antigen-specific immunotherapies to treat the disease.

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The existence of autoimmune diseases in humans has been known for almost 100 years. Currently, autoimmune pathogenesis has been attributed to more than 40 human illnesses, yet it is still not clear what immune abnormalities conclusively prove underlying autoimmune pathogenesis. Autoreactivity, by definition, designates a specific adaptive immune response against self-antigens. Normally, there is tolerance to self-antigens, achieved by physical deletion or functional silencing of specific T and B cells. How tolerance fails and autoimmune disorders arise remain incompletely understood. Organ-specific autoimmune diseases are directed primarily at the autoantigens of particular tissues: insulin-producing β cells in type I diabetes, platelets in autoimmune thrombocytopenic purpura (AITP), and red blood cells (RBCs) in autoimmune hemolytic anemia (AIHA). These two hematologic disorders are mediated by autoantibodies and, as with all organ-specific autoantibodies, the antibody production is dependent on T-cell activation. This review will outline current concepts of autoimmunity and evidence for the etiology of AIHA.

Normal Immunity

The initiation of a humoral immune response to a foreign antigen is a complex biologic association of many cell types and their secreted products. Response occurs when an antigen, such as a cell surface glycoprotein, first interacts with an antigen-presenting cell (APC).[1] APCs are generally major histocompatibility complex (MHC) class II–positive macrophages or dendritic cells and, in certain instances, B cells. After internalization by the APC, the antigen is processed into smaller antigenic fragments by proteolytic degradation.[1] The antigenic peptides are then transported to the cell membrane of the APC, where they are re-expressed in conjunction with MHC-encoded class II molecules for presentation to antigen-specific T-helper (Th) lymphocytes.[1,2] When the MHC–peptide complexes are recognized with sufficient affinity by T-cell receptors (TcRs) on a $CD4^+$ Th cell, antigen-specific signal 1 is triggered and initiates a set of coordinated molecular events in both the APC and the T cell that culminate as signal 2 (costimulation) and full T-cell activation (such as CD40-mediated upregulation of B7 molecules on the APCs).[2] These T-cell/APC events can stimulate antigen-primed B cells to differentiate into plasma cells and secrete antigen-specific antibodies. Thus, any defect in or abnormal stimulation of antigen-specific Th cells can significantly alter the course of an immune response. Th cells are vital in determining whether antibodies will be generated against a foreign antigen and, once generated, in regulating the response by stimulating antibody affinity maturation, regulatory T cells, and secretion of cytokines.

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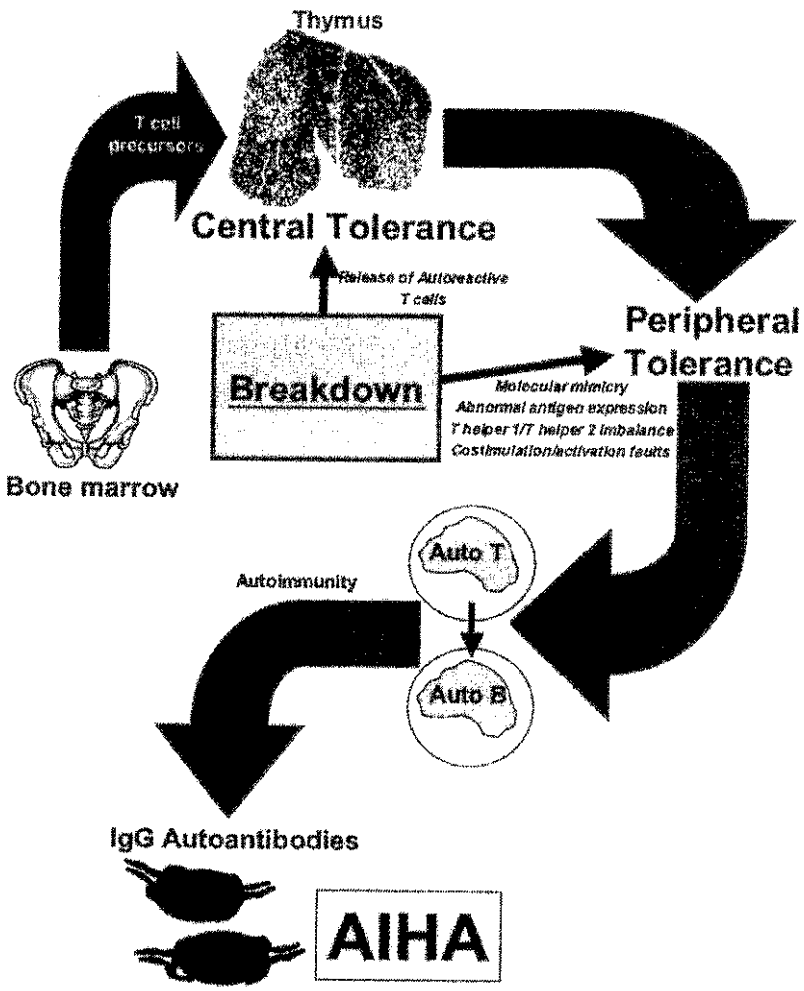

Figure 1 Central and peripheral tolerance. The pathways that lead to the evasion of tolerance are boxed.

T-Cell Tolerance

Normally, a host does not mount an immune response against its own antigens; it behaves as if it is immunologically "ignorant" or tolerant. Immunologic tolerance is the acquisition of unresponsiveness to self-antigens and is essential for the preservation of the organism. The major theories (Figure 1) postulate that T-cell tolerance (Figure 1) is induced either by (1) clonal deletion of high-affinity self-reactive T cells within the thymus, called "central tolerance"; or (2) by autoreactive T cells being deleted or rendered anergic by specific and nonspecific mechanisms in the extrathymic milieu, or "peripheral tolerance."

Central Tolerance

Thymic selection is the central process by which the generation of TcR diversity against self-antigens is limited. As a developmental process, T cells with a biased repertoire are selected for export into the periphery.[3] In the course of positive selection, T cells that interact only weakly with self-peptides presented in the context of MHC molecules are chosen, while those that do not effectively interact with MHC–peptide complexes die "by neglect." In addition, interactions with high avidity result in elimination by negative selection and constitute the basis for "central tolerance." Central tolerance prevents widespread autoimmunity, preferentially selecting T cells with specificity for antigens not expressed in thymic epithelium for export into the periphery. However, central tolerance is not complete and a sizable pool of T cells with intermediate avidity can escape negative selection: they constitute the majority of autoreactive T cells found in the peripheral immune system. While the presence of these autoreactive T cells is considered physiologic, they are usually not activated and exhibit a "naive" phenotype. Only an encounter under appropriate stimulatory conditions can lead to their full activation in the periphery. Autoreactive T cells are potentially very dangerous and capable of initiating organ-specific autoimmunity should they recognize their autoantigen in a defined tissue.

Peripheral Tolerance

The presence of autoreactive T cells in the periphery suggests that autoimmunity should occur frequently, but there are

several mechanisms that maintain tolerance in the periphery. For example, naive T cells triggered only by a signal through the TcR lose their ability to proliferate and become anergic. However, the presence of certain cytokines or costimulatory interactions can avoid the induction of anergy or reverse an anergic state. In addition, T cells that are activated in the periphery eventually undergo activation-induced cell death (AICD).[4] AICD is thought essential for the down-modulation of the normal immune responses and the re-establishment of immune homeostasis. Impairment of AICD may lead to continued immune activation and generalized autoreactivity. For CD4 lymphocytes, AICD appears to be Fas/Fas-L–dependent, but it is not clear which interactions control AICD in CD8 cells. Furthermore, molecules that can deliver specific negative signals, such CTLA-4, are involved in the "turning off" of antigen-specific T cells. Of interest with respect to AIHA, a point mutation within the *CTLA-4* gene is a major predisposing factor to the development of autoantibodies.[5] Other factors, such as regulatory T lymphocytes and APCs, may also play important roles in maintaining peripheral tolerance.[6]

Factors Affecting Initiation of Autoimmunity

Several factors affect the initiation of autoimmune responses: the nature of the antigenic stimulation, the genetic composition of the host, the environment to which the host is exposed, and the immune regulatory circuits utilized by the host.

Autoantigens

A major challenge in most human autoimmune disorders is the identification of initiating autoantigen(s). Candidate autoantigens have been identified in several autoimmune diseases, including type 1 diabetes, multiple sclerosis, rheumatoid arthritis, AITP, and AIHA. In AIHA, for example, autoantigenic T-cell epitopes have recently been mapped for the RhD autoantigen.[7] Whether any of these autoantigenic sites are actually involved in initiation of the disease remains unclear and controversial. Understanding the features that constitute an ideal candidate autoantigen and the parameters by which these antigens are identified as the initiating factor is critical to preventive or therapeutic interventions.

Genetic Relationships

Many lines of evidence indicate the association of organ-specific autoimmune diseases with certain haplotypes of the human leukocyte antigen (HLA) complex. In general, MHC class I or class II genes predispose an individual to a certain autoimmune disease by, for example, enhanced presentation of exogenous pathogenic peptides in the periphery or improper presentation of self-derived peptides in the thymus. For example, the human HLA-DQ6 molecule has been associated with AIHA.[8] In addition, in a murine model of AIHA the production of RBC autoantibodies is under multigenic control outside of the MHC.[9] For most organ-specific autoimmune disorders, the genetic links are complex, not absolute, and many susceptibility and resistance genes act in concert to modulate autoimmunity.

Environmental Causes

For many years, viral infections have been proposed as potential triggers of autoimmunity in susceptible individuals because of their capacity to directly infect target tissues and induce strong inflammatory responses and immune activation. While the association between viral infections and organ-specific autoimmune disorders is intriguing, it has been difficult to demonstrate a causative role for specific viruses in human autoimmune diseases. Acute AITP may be the result of tolerance breakdown due to antigenic mimicry.[10] Antigenic mimicry is one of the oldest and most prevalent theories of tolerance breakdown resulting in autoimmunity. Molecular structures on infectious or environmental agents have similarities to host antigenic structures and stimulate an immune response cross-reactive with self. Many children with acute AITP spontaneously remit without therapy, as a putative infectious agent is cleared and antibodies either disappear or mature in affinity to be more specific to the infectious agent, and the antiplatelet reactivity is lost.

Other inflammatory stimuli may similarly trigger or enhance autoimmunity. The gut harbors thousands of different bacterial strains and viral infections. The mucosal lining is permeable for various molecules and nutrients and is a site of significant interaction with the environment, which in some circumstances may predispose individuals to autoimmune attack.[11] The commensal flora also are critical to maintaining proper immune activation and function. The balance of these factors may determine whether autoimmunity occurs.

Regulatory Circuits in Autoimmunity

Once Th cells are activated, their responses can be generally distinguished by their secreted cytokine products.[12] A Th1 response is characterized primarily by the presence of interleukin (IL)-2, interferon (IFN)-γ, granulocyte-macrophage colony-stimulating factor (GM-CSF0, and tumor necrosis factor (TNF)-α and is associated with delayed-type hypersensitivity reactions and the synthesis of primarily complement-fixing IgG isotypes. Th2 responses produce IL-4, IL-5, IL-6, IL-10, and IL-13 and are superior in mediating non–complement-fixing IgG and particularly IgE synthesis. A third type of Th response, Th0, is thought to be generated by cells less differentiated than those mediating Th1 and Th2 responses, since many or all of the Th1/Th2 cytokines are present. These cytokine responses are the result of coordinated efforts between APC and Th cells. The nature of the cytokine response is ultimately responsible for the particular outcome of an immune response, as for generating a particular isotype of antibody to remove the initial antigenic insult. Th1 responses are generally associated with active organ-specific autoimmunity, and resolution of autoimmunity is thought to be related to Th2 responses.[13]

Cytokines and chemokines play a central role in initiating and maintaining autoimmune pathogenesis, and they consti-

tute a multitude of positive, as well as negative, feedback loops that can become deregulated.[13] For example, certain cytokines can negatively or positively influence the production of other cytokines (as in the Th1/Th2 paradigm) and thus determine the balance between pro- and anti-inflammatory factors in the local environment.[13] Cytokine networks operate redundantly, and cytokines and chemokines share common receptors and are likely mediators of bystander activation processes. These pathways may offer effective targets for therapeutic cytokine/chemokine-blocking antibodies.

Apoptosis

Stimulation of the immune system is followed by a process that reverses activation and re-establishes homeostatic baseline levels of immunity.[4] In the absence of such regulatory mechanisms, immune responses escalate and excessive autoimmune pathology may occur. Thus, activation-induced programmed cell death is believed to play an important role in regulating autoimmunity, and defects of apoptosis are related to autoimmunity, including AIHA.[14-18] For example, defective apoptosis of autoaggressive T cells expressing IL-2 receptors may play a role in initiating AIHA pathogenesis. Thus, an ongoing autoimmune process can be viewed as a fine-tuned but fragile equilibrium of aggressive and regulatory components: the precise activation kinetics and survival times of all lymphocyte types implicated in the process will determine the outcome.

Kinetics

The pathogenesis of autoimmunity is related to the kinetics of immune responses. The pathophysiologic or beneficial effect of a lymphocyte population depends not only on its specificity, activation state, and effector functions, but is also a function of timing—the phase of an ongoing disease process in which it is present. Inflammatory cytokines such as IFN-γ or TNF-α exhibit opposing effects in type 1 diabetes, depending on when they are generated.[19] Early expression enhances islet destruction and disease development, whereas late expression ameliorates disease by inducing apoptosis of autoaggressive cells. Kinetic issues may constitute a major obstacle for successful immune therapeutic intervention, because they preclude the use of specific blocking agents or administration of cytokines without precise knowledge of their kinetically differential role in the disease process. Treatments will likely have to be individualized for antigen-specific immune-based interventions.

Autoimmune Hemolytic Anemia

AIHA is characterized by the production of antibodies directed against self RBCs. Since the autoantibodies usually are directed against highly prevalent antigens, they often exhibit reactivity against allogeneic RBCs as well. The etiology of most RBC autoantibodies is not well understood. Idiopathic or primary AIHA shows no apparent association with an underlying disorder. Given the frequent association between AIHA and other autoimmune disorders, however, generalized immune system dysfunction, as discussed above, likely plays a role, and the relationship between AIHA and lymphoproliferative disorders and other neoplasms supports the concept that failed immune surveillance may underlie AIHA. Recent studies on animal and human AIHA suggest that loss of immunologic tolerance to RBC self-antigens may arise by different, nonmutually exclusive mechanisms: (1) ignorance against RBC self-antigens, (2) molecular mimicry, (3) polyclonal T- and/or B-cell activation, (4) errors in central or peripheral tolerance, and (5) immunoregulatory disorders including cytokine network alteration.

In some patients with AIHA, experiments employing stimulation of peripheral blood mononuclear cells (PBMC) by synthetic Rh peptides suggest that ignorant T- and/or B-cell clones can recognize cryptic RBC self-antigens. Crypticity may be an important characteristic of epitopes recognized by autoreactive T cells.[20] T cells specific for cryptic determinants are part of the normal T-cell repertoire but normally do not encounter the relevant antigenic peptides in the periphery. If the cryptic self-determinant is presented, for example as a result of conformational change, increased concentration, or protease action, these T cells may become activated and autoaggressive, resulting in autoimmune disease. The key, unanswered question, is how normally cryptic epitopes become visible to the immune system and produce a sustained pathogenic response.

AIHA following bacterial or viral infection seems to result from the polyclonal T- and/or B-cell activation against foreign antigens which mimic protein or carbohydrate epitopes on RBC. Polyclonal activation of host B-cell clones by donor alloreactive T cells may be responsible for the AIHA seen in chronic graft-versus-host disease. With regard to loss of tolerance, experiments using mouse cell lines expressing a transgene with autoantibody activity against murine RBC showed that nondeleted peripheral B-cell clones may produce RBC autoantibodies. In humans, a genetic defect of Fas/Fas-L–mediated autoreactive lymphocyte apoptosis may be associated with AIHA.[14] Immunoregulatory disorders due to depletion of $CD4^+CD25^+$ T-cell or Th1/Th2 cytokine imbalance also may induce autoimmune diseases. In murine and human AIHA, there is an increased production of Th2 cytokines (IL-4 and IL-10), but IFN-γ production is reduced; in human AIHA downregulation of IL-12 suggests that an imbalance of the IL-10/IL-12 immunoregulatory circuit may facilitate RBC autoantibody production.

Cold Versus Warm Autoantibodies

Pathologic cold agglutinins are produced either in response to infection or by paraneoplastic or neoplastic growth of a single immunocyte clone. In either case, they usually share the same immunochemical characteristics and polysaccharide specificities. While infection may elicit transient cold agglutinin syndrome, lymphoproliferative disorders typically lead to a more chronic disease. The cellular origin of cold-reactive autoantibodies in AIHA has been elucidated by model systems of Epstein-Barr virus–transformed cell lines

secreting anti-I, anti-I, and anti-Pr_2 pathogenic autoantibodies. In about 40% of patients, a circulating B-cell clone could be identified with a distinctive karyotypic marker, such as trisomy 3, trisomy 12, or 48,XX,+3,+12; this chromosomal aberrancy was associated with chronic idiopathic cold agglutinin syndrome as well as with monoclonal cold agglutinins secondary to a B-cell neoplasm.[21,22] Furthermore, the secreted monoclonal antibodies had the same serologic specificity and isoelectric focusing spectrotype. Thus, the monoclonal cold agglutinins were derived from the associated (pre-)neoplastic B-cell populations found in patients.[21,22] It has not been possible as yet to generate relevant stable in vitro cell lines to provide a continuous source of homogeneous antibody for antigen-binding and structural analyses.

Cold agglutinins increase during viral or bacterial infections; the typical infectious etiology is mycoplasma pneumonia or infectious mononucleosis in an adolescent or a young adult. The I/i carbohydrate structure is present both on RBCs and leukocytes, as well as on macrophages, platelets—and bacteria. The sialylated form on glycolipids of the I/i antigens expressed on the RBC surface may act as a *Mycoplasma pneumoniae* receptor, and the increase in cold agglutinin levels induced by mycoplasma infection may be due to common antigenic epitopes on RBCs and mycoplasma. Some evidence indicates that the binding of mycoplasma to its receptor could be indirectly responsible for cold agglutinin-producing B-cell clone activation. Other infections associated with cold agglutinin syndrome include adenovirus, cytomegalovirus (CMV), influenza viruses, varicella zoster virus (VZV), human immunodeficiency virus (HIV), *Escherichia coli*, *Listeria monocytogenes*, and *T pallidum*. AIHA and infections are also related in paroxysmal cold hemoglobinuria (PCH); while both idiopathic PCH and PCH secondary to syphilis are chronic processes that have become increasingly uncommon, today most cases of PCH have an acute transient pathology secondary to infection, often antecedent upper respiratory infection, the cause of which may not be identified. Agents associated with PCH include measles, mumps, CMV, VZV, adenovirus, influenza A, *Mycoplasma pneumoniae*, *Hemphilus influenzae*, and *E coli*, and possibly also vaccination for measles. In PCH, the autoantibody is a cold-reacting IgG, always polyclonal, that reacts with the P antigen, a polysaccharide usually fixed to a ceramide moiety.

With regard to structural studies of cold agglutinins, cross-reactive idiotypes have been found among RBC autoantibodies[23]; this cross-reactivity appeared to be restricted to RBC autoantibodies with similar specificity. One monoclonal antiidiotypic antibody (9G4) recognized an idiotypic determinant present on the heavy chains of both anti-I and anti-i cold agglutinin as well as on neoplastic B cells secreting cold agglutinin.[24] This idiotype was found in 47 of 48 pathogenic anti-I/i cold agglutinins, and both the anti-I and anti-i cold agglutinins derived from a distinct subset of V_H4 family genes, $V_H4.21$.[24-28] The remarkable finding of restriction in the variable region genes utilized for the anti-I/i heavy chains, along with diversification in the associated light chain variable-region gene usage, suggests a model for the relative contribution of heavy and light chains to antigen binding. Practically, the similarities in idiotypic structure among cold agglutinin heavy chain variable regions, and the ability to generate antiidiotypic antibodies specific for these structures, suggests exciting potential therapeutic applications for these reagents to downregulate autoantibody production. In contrast to the structural uniformity of anti-I cold agglutinins, substantial structural differences have been shown in anti-Pr_2 cold agglutinins[29] and, in the Pr_2-specific response, self-antigen may play a role in the pathogenesis of the autoreactive B-cell neoplasm.[30]

IgM autoantibodies generally react with polysaccharide antigens on the RBC surface, and IgG warm autoantibodies generally react with protein antigens on the RBC surface. IgG autoantibodies are typically panagglutinins, reacting with all RBCs, and by immunoblotting they may react with Rh antigens, membrane protein band 4.1, protein band 3, and glycophorin A as universal RBC targets. The association of warm-type AIHA with systemic autoimmune disorders implies that these RBC autoantibodies, in contrast to clonal cold-reactive autoantibodies, might arise from polyclonal activation rather than in response to activation by specific (self-)antigen.[31] Furthermore, warm-reacting autoantibodies, when associated with a clonal B-cell lymphoproliferative disorder, are not secreted by the neoplastic B: they differ in isotype from immunoglobulin expressed by the malignant B cells. As an alternative etiology, warm-reacting IgG RBC autoantibody might arise from immunologic network interactions involving anti-idiotypes.[32]

Autoantigens and Environmental Factors in AIHA

The major antigenic sites involved in warm AIHA are the Rh complex and glycophorin. For the proteins of the Rh complex, the epitope against which the autoantibody is directed is most commonly nonpolymorphic and present in all individuals (except those with the rare Rh null phenotype). In some cases, the epitope may include some or all of the polymorphic portion of the protein that defines a specific antigen; more rarely, it is directed to a specific blood group antigen. Equally frequently, antibodies in AIHA also may be specific for antigens on the major glycoproteins of the RBC membrane. The epitopes are usually amino acid sequences in the nonpolymorphic portions of the molecule and, rarely, epitopes on other proteins of the RBC membrane.

As described above, different bacterial or viral molecules can act as B-cell stimulators, and AIHA is often a complication of viral infections. The role of viruses in the etiology of autoimmunity has been proposed to be secondary to antigenic mimicry, production of antiidiotypic antibodies against viral receptors, antigenic epitope modification, and B-cell polyclonal activation. After intracerebral inoculation of lymphocytic choriomeningitis (LCM) virus, mice developed AIHA[33]; the immune hemolysis was strongly reduced by treatment with CD4 antibody, suggesting that the virus-induced AIHA was a Th-dependent autoimmune event.

As discussed elsewhere in this issue, drugs may be associated with AIHA,[34] as a result of several types of interaction

among drug, antibodies, and RBC membrane components. Alpha methyldopa is the prototypical drug operating by the induction of autoantibodies; these are typically panreactive, although specificities have been described. Other drugs that can induce autoantibodies include levodopa, mefenamic acid, procainamide, and diclofenac; for these, the antibody reacts with a normal membrane component and the epitope does not involve the presence of a drug—they function as true autoantibodies. The drug may alter antigens on the RBC, resulting in production of antibody that cross-reacts with the unaltered antigen. While the exact cause of methyldopa-induced AIHA has not been established, it is likely that the drug, a known protein-reactive "tanning" agent, alters one of the components of the Rh complex, rendering it antigenic; the resultant antibody is able to cross-react with the normal, unaltered epitope.

Transfusion and AIHA Autoantibodies

The most obvious association of autoantibody formation following transfusion is seen in sickle cell disease and thalassemia: 9% of sickle cell disease patients develop RBC autoantibodies.[35] The phenomenon of RBC autoantibody formation in association with blood transfusion is not well understood. Alloantibodies may bind to transfused red cells and cause conformational changes in the red cell antigenic epitopes, leading to stimulation of autoantibody formation.[36] Alternatively, since antibody formation has been observed particularly in multitransfused sickle cell disease, some patients may simply have a predisposition to develop RBC autoantibodies, perhaps because of an overall dysfunction of their immune systems.[36,37] The development of RBC cold autoagglutinins was observed in animals following repeated injection of red cells and has occasionally been seen in humans associated with delayed hemolytic transfusion reactions. The development of autoantibodies also occurs after an episode of RBC destruction by passively administered antibodies and following intensive plasma exchange. Most recently, two patients with AIHA suspected to be associated with previous blood transfusions were described, again drawing attention to a phenomenon that has been long recognized but to which little attention has been paid.[38] As long ago as 1918, Rous and Robertson showed that rabbits who had received small transfusions of allogeneic blood developed strong cold autoagglutinins.[39] IgG warm autoantibodies sometimes also form in transfused animals: chimpanzees developed autoagglutinins after immunization with human RBCs and positive direct antiglobulin tests (DATs) develop in rabbits and mice after injection of allogeneic blood. Worlledge[40] in a review on interpretation of positive DATs stated that persistent alloimmunization may lead eventually to autoimmunization, even though compatible RBCs are given. Positive DATs associated with delayed hemolytic transfusion reactions may remain positive for up to 300 days, well beyond the presence of transfused RBCs in the circulation, leading to the hypothesis that the original alloantibody developed autoantibody characteristics.[41,42] There are changes in self-reactive antibody repertoires of plasma IgM and IgG after transfusion, independent of a specific immune response to RBC antigens.[43] Using phage display technology, RNA-derived B-lymphocyte repertoires from D^+ donors have anti-D reactivity resembling a typical cold antibody, consistent with other observations that the B-cell repertoire contains a level of self-reactivity.[44] AIHA may sometimes occur because lymphocytes from previous transfusions survive and create a graft-versus-host situation: post-transfusion survival of foreign leukocytes, even in immunologically normal individuals, is now well established, and there is a strong association of such microchimerism with autoimmune disease.[45] All these data suggest that RBC autoantibody development in transfused individuals may be the result of either simple cross-reactivity of the alloantibodies with self RBC antigens or due to determinant spreading during the maturation of the alloantibody response.

Immune Deviation in AIHA

There is in vitro and in vivo experimental evidence to suggest that quiescent T and/or B cells specific for self-antigens may be activated, if antigen presentation and costimulation are adequate.[46] Polyclonal activation occurs in patients with AIHA. RBC autoantibodies were present in both healthy individuals and AIHA patients, but the autoantibody levels and specificities were higher and skewed in the latter group.[47] Other research indicates involvement of the B-1 subpopulation of B lymphocytes in AIHA[48,49]; unlike conventional self-reactive B cells in the periphery and bone marrow, self-reactive B-1 cells in the peritoneal cavity are separated from red cells and may therefore escape clonal deletion.

Dacie[50] suggested that acute transient AIHA may result from RBC antibodies developing as a result of microbial infection in patients with an unusual ability to develop antibodies, immunologic self-tolerance being perhaps broken as a result of the invading organism modifying the antigenicity of RBC antigens or by unmasking antigens that were not normally cryptic. In chronic AIHA, self-tolerance may perhaps be broken by a failure of T-lymphocyte surveillance or an abnormal T suppressor to T helper ratio, and IgA deficiency also may play a part. Genetic factors may be significant, creating a propensity to form antibody. In chronic cold agglutinin disease, the underlying clonal lymphoproliferative disorder with somatic mutation of unknown causation may result in the unrestrained proliferation of immunocytes dedicated to the production of cold autoantibodies.

In support of the hypothesis of self-ignorance in human AIHA, synthetic peptides corresponding to Rh polypeptide sequence, the most frequent target for autoantibodies in human warm-type AIHA, were tested in vitro for their ability to stimulate normal T cells.[50,51] Multiple peptides provoked T-cell activation, and their proliferation could be blocked by anti–HLA-DR antibodies. Autoreactive T cells in AIHA may not be deleted but are anergic to autologous Rh polypeptides and thus immunologically ignorant against RBC self-antigen.[51,52] In addition, there are experimental data that autoantibody production in some cases of AIHA is caused by the activation of class II–restricted helper T cells specific for cryptic Rh epitopes[53]; these autoreactive T cells seem to escape

clonal deletion and anergy during the induction of self-tolerance and remain quiescent, even if the autoantigens they recognize are present. In AIHA, ignorant T cells could be activated through independent events, such as persistent stimulation of cross-reactive environmental antigens or Th1 cytokine-induced changes in autoantigen processing that trigger the presentation of cryptic epitopes.

In patients with AIHA, background levels of T-cell proliferation in vitro were increased more than twofold that of controls, consistent with a state of hyperactivation.[54] However, after anti-CD3 stimulation, the proliferative response of the T cells was markedly reduced, while IL-10 and IL-2 production was increased.[54] Basal production of IL-4 and TNF-α also were increased in cultures of PBMC from patients with AIHA.[55] Taken together, the data suggest that in human AIHA, cytokine abnormalities exist and relate to the Th1/Th2 axis; cytokine responses may contribute to the immunopathogenesis of AIHA. More specifically, IL-10 may induce RBC autoimmunity and maintain the disease by continuous autoantibody production.[54-56]

Animal Models of AIHA

The mouse strains NZB and NZB/NZW spontaneously develop a complex autoimmune syndrome including AIHA; they have been extensively studied to identify immunologic factors contributing to the autoimmune onset of AIHA.[9,31,48,57-63] NZB mice have provided experimental evidence to support an antigen induction model of AIHA: RBC membrane band 3, a red cell anion exchange protein, appears to be the major antigen for RBC autoantibodies. Although not all autoantibodies binding to band 3 produce pathologic effects, in NZB mice with band 3–reactive CD4 T cells, pathogenic autoantibodies are found.[57] Similarly, in humans, many AIHA patients expressed Th cells which bind to the Rh antigen on human RBCs; B cells, however, were found not to react with the same epitopes on the Rh antigen, which is recognized by the Th cells.[52,53] Changes induced in MHC II autoantigen processing might result in the presentation of previously cryptic epitopes to which naive Rh-reactive T cells respond.[59,63] This hypothesis was supported by the finding of another autoantibody in NZB mice for murine RBCs, which binds to a partially masked epitope when the RBCs are treated with protease to enhance expression of the epitope.[57] The Th1 predominant response to band 3 elicited IFN-γ production, and this cytokine may promote presentation of the cryptic epitopes. How a self-reactive T cell might escape clonal deletion to respond to the self-antigen is still not yet fully understood.

That animal models autoimmune diseases can be induced by a Th1/Th2 cell cytokine imbalance may have relevance to the pathogenesis of human AIHA. In NZB/W mice and AIHA there is increased production of Th2 cytokine (IL-4 and IL-10) but IFN-γ is reduced, suggesting that an imbalance of cytokine immunoregulatory circuits may facilitate RBC autoantibody production.[46,54] Involvement of Th1 cells has been inferred in the general autoimmune syndrome in NZB/W mice, and IFN-γ, a Th1 cytokine, can promote B-cell maturation and pathologic antibody generation, accelerating development of the syndrome. Continuous administration of anti–IL-10 antibodies delays the onset of autoimmunity in NZB/W mice, due to increase of TNF-α, whereas IL-10 administration accelerates its onset.[60] Furthermore, administration of IL-5– and IL-10–activated peritoneal B1 cells can induce AIHA in transgenic mice.[61,63]

Self-reactive autoantibodies exist in the peripheral lymphoid organs of normal animals. The simplest explanation is that the B cells that make the autoantibodies already exist in the peripheral lymphoid compartment, awaiting the proper stimulus. Transgenic mouse models have provided convincing evidence of the existence of such B cells. When the V genes that came from an actual RBC autoantibody, originally obtained from a NZB mouse with AIHA, were expressed in transgenic normal mice, central deletion was seen in most of the animals, but many also had some residual autoreactive B cells in spleen and lymph nodes, and some otherwise normal mice even developed frank AIHA. Central self-tolerance therefore is not completely efficient, even in a non-autoimmune mouse, and some autoreactive B cells can be stimulated to cause disease. Oral administration of lipopolysaccharides (LPS) to HL mice with H and L chains derived from NZB mice induced peritoneal B1-cell secretion of RBC autoantibodies in the gut lumen and resulted in AIHA.[48,63] Th2 cells could cause AIHA by IL-5 and IL-10 induction of autoantibody-secreting B1 cells. Furthermore, elimination of B1 cells not only reduces the amount of IgM autoantibody but also the amount of IgG autoantibody, demonstrating B1 cell involvement in IgG, as well as IgM, production.[49]

Conclusions

The immunopathogenic etiology of AIHA remains poorly understood. Generalized dysfunction of the immune system and immune surveillance likely are involved. Disruption of any of the processes or control points that maintain a balance between tolerance of RBC self antigens and the need to respond to foreign antigens may be a cause of the onset of AIHA. While RBC autoantibodies may arise from malignant B-cell clones, in AIHA the autoantibodies are generally polyclonal. Superimposed genetic and environmental factors also play a role in the production of autoantibodies in AIHA. More research to clearly map the underlying immune defects in AIHA may lead to the development of more antigen-specific therapies for AIHA.

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