In early July 2001 a small meeting was held in Baltimore on the associations of lymphomas with autoimmune conditions. Topics ranged from the effects of defective apoptosis to questions of antigen-drive lymphoproliferation.

Autoimmunity and lymphoma: tribulations of B cells

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Since the early 1950s, when autoimmunity began to emerge as a recognized disease process, numerous studies linked several different autoimmune diseases with benign and malignant lymphoproliferation in humans as well as animal models[1–4]. To explore these apparent associations in the light of a new understanding of the molecular genetics of the immune response, a workshop was recently convened in Baltimore, Maryland, to consider some relevant questions. (i) Which associations are real and which are the most significant? (ii) What is the involvement of autologous or exogenous antigen? (iii) Is autoimmunity associated with B cell or T cell lymphomas? (iv) Is there any practical value to understanding the autoimmunity-lymphoma association? (v) Can we gain fundamental insights by examining this? The conference brought together basic and clinical investigators from the disciplines of immunology, hematology, oncology, molecular biology and genetics. The major themes of the meeting were the role of defective apoptosis, immunoglobulin gene rearrangement, translocation and hypermutation, sustained antigen-driven lymphoproliferation by endogenous or exogenous antigens and Sjögren's syndrome as a clinical paradigm of autoimmunity and lymphomagenesis.

Strength of association

Existing clinical data on the autoimmunity-lymphoma association are rather meager because many of the clinical studies have been anecdotal case collections based on a "numerator" (occurrence of disease) without a "denominator" (a well studied at-risk population with either a given autoimmune disease or lymphoma). However, adequate studies establish strong associations between B cell lymphomas and Sjögren's syndrome[5,6], autoimmune thyroiditis[7] and autoimmune hemolytic anemia[8]. There are weaker associations between B cell lymphomas and systemic lupus erythematosus (SLE)[9,10] and rheumatoid arthritis[11]; however, these may be due to the possible confounding effects of the treatments used. There is a highly likely (but yet undefined) association between T cell lymphoma and celiac disease[12], and with tropical spastic paraparesis associated with human T lymphotrophic virus 1 (HTLV-1)[13]. For most examples of association, autoimmune disease precedes lymphoma, although for the classical association between autoimmune blood diseases and lymphoma (or chronic lymphocytic leukemia) it is uncertain which comes first.

Apoptosis

The role of defective apoptosis in the genesis of lymphoproliferation, autoimmunity and lymphoma was dramatically illustrated by the autoimmune lymphoproliferative syndrome (ALPS) of childhood, the human equivalent of the earlier recognized mouse model, MRL[lpr14]. ALPS is the result of dominant heterozygous inheritance of a mutated (inactive) gene, *TNFRSF6*, which encodes the transmembrane

protein Fas (also known as CD95), a major mediator of lymphocyte apoptosis. The Fas mutations predominantly involve the intracellular (death) domain of Fas. Extensive, diffuse lymphadenopathy is often accompanied by autoimmune hemolytic anemia, neutropenia, thrombocytopenia and hypergammaglobulinemia. Among cases studied at the US National Institutes of Health (NIH), lymphoma occurred in 6 of 46 individuals, 13% of patients, usually long after the onset of ALPS at intervals from 6–48 years (Elaine Jaffey, Bethesda, MD). The benign lymphoproliferation includes distinct cell types: "double negative" CD4-CD8-T lymphocytes with an αβ T cell receptor (TCR) and CD5+ (B-1) B lymphocytes[15]. ALPS lymphomas are almost exclusively of B cell origin, with diverse histologies that include Hodgkin's disease variants, T cell–rich large B cell lymphomas, Burkitt lymphomas and follicular lymphomas. Gene penetrance is variable: not all carriers of intracellular Fas mutations develop ALPS, suggesting that mutant Fas operates on a background of other (unknown) genetic or environmental influences (Michael Lenardo, Bethesda, MD). Epstein-Barr virus (EBV) has been considered as an environmental candidate because lymphoma cells in three of six cases examined at NIH showed EBV expression. Experience so far shows that Fas mutation is not a feature of spontaneous human autoimmune disease, but this needs more extensive investigation. Spontaneous lymphoma can occur on a background of Fas mutations, as judged by the occurrence of such mutations in about 16% of lymphomas studied, which are mostly extranodal (Klaus Rajewsky, Cologne, Germany).

Sustained antigen drive

The second mechanism considered was sustained antigen drive, although much of the evidence presented at the conference incriminated microbial infection rather than autoantigens. Thus mucosa-associated lymphoid tissue (MALT) lymphoma of the stomach is associated with gastritis due to colonization with *Helicobacter pylori*[16] rather than gastritis due to autoimmunity (that is, pernicious anemia-type gastritis). The host mounts an on-going but noneradicating immune response to *H. pylori* that, with T cell stimulation, generates an actively proliferating B cell population. The ensuing MALT lymphoma will regress up to a certain point if *H. pylori* is eradicated. However, acquisition of particular chromosomal translocations among reactive B cells are lymphomagenic. For example, the most commonly found translocation, t(11;18) (q21;q21), results fusion between the gene encoding AP12 (an apoptosis inhibitor) and the gene encoding MALT-1, which encodes a paracaspase that indirectly activates NF-κB. The less common t(1;14) (p22;q32) translocation causes aberrant expression of Bcl10[17,18], a protein that binds to MALT-1, which again leads to activation of NF-κB. Either way, the MALT-1 lymphoma
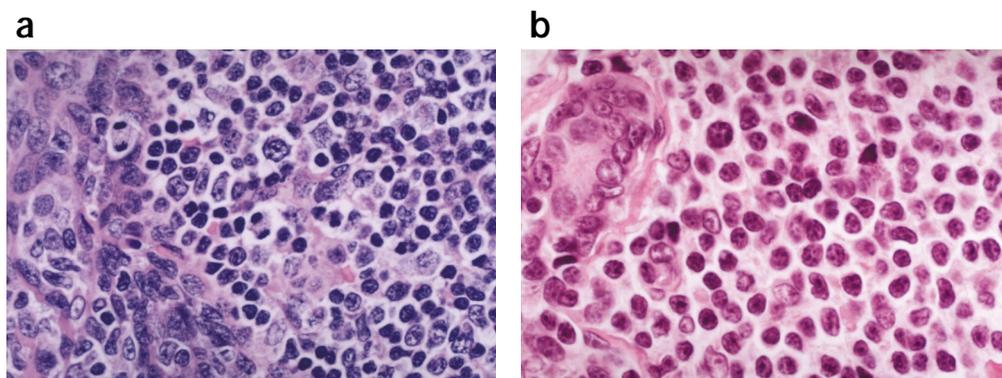
Figure 1. High-magnification micrographs that compare and contrast florid lymphoepithelial sialadenitis (LESA) and a low-grade extranodal marginal zone lymphoma of mucosal-associated lymphoid tissue (MALT) type in a parotoid gland. (**a**) LESA is characterized by a polymorphous lymphocytic population, which includes scattered immunoblasts. On the left-hand side, an epimyoepithelial island is invaded by the reactive lymphocytes. (**b**) Low-grade malignant MALT lymphoma in a parotid salivary gland; the lymphocytes are monomorphous with distinct monocytoid features. (Original magnigication: ×240).

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loses dependence on *H. pylori* and becomes autonomous, with further possible oncogenic activity provided by inactivation of the p53 and p16 tumor suppressor genes. Sustained antigen drive is exemplified by hepatitis C virus (HCV) infection of the liver and other tissues. A well known immunological complication of HCV infection is mixed cryoglobulinemia (MC) with vasculitis in which up to 86% of the cryoglobulins contain HCV RNA[19] . Monoclonal B cell expansion evolves through lymphoproliferative disease (in liver and bone marrow particularly) to B cell lymphoma with types that include MALT-derived—nodal or splenic—or diffuse large B cell lymphoma. Again, interference with apoptosis is implicated, as patients show increased expression of Bcl-2 by liver and bone marrow cells and t(14;18) Bcl-2 recombinations in peripheral blood lymphocytes[20]. Geographic and ethnic differences in the frequency of the association of HCV with B cell lymphoproliferative disorders suggests the participation of other undefined genetic and/or environmental cofactors (Clodovio Ferri, Pisa, Italy).

Further evidence relating to antigen drive was derived from the clonal lymphoproliferation expressed as B cell chronic lymphatic leukemia (B-CLL), which is a well known accompaniment of autoimmunity that affects blood cells[21]. It was based on analysis of the DNA sequences of rearranged and expressed variable heavy (V_H) and variable light (V_L) genes from normal CD5^{+} B cells and B-CLL cells, classified according to whether they were immunoglobulin M–expressing (IgM^{+}) or IgG^{+} cells. There were distinct differences in V gene use and mutation among normal CD5^{+} B cells and between IgM^{+} and IgG^{+} B-CLL cells. This was interpreted as selection for V genes that reflected reactivity with autoantigens (unmutated Ig V_H1-69–expressing cases) or xenoantigens (mutated Ig V_H3-07–expressing cases). However, the presumed autoantigens or xenoantigens were not identified. The interesting point was that these subgroups of B-CLL patients had quite different outcomes and responses to treatment, with generally poor outcomes in the autoreactive B-CLL group and good outcomes in the xenoreactive B-CLL group (Nicholas Chiorazzi, New York, USA).

A different cause for lymphocyte persistence, but with similar outcomes (autoimmunity and lymphoma), is illustrated by the forced overproduction of B lymphocyte stimulators (members of the BLyS family), which act during maturation of B lymphocytes. Mice carrying a transgene for B cell activating factor (BAFF), a BLyS family member, become highly susceptible to the lymphoproliferation-autoimmunity-lymphoma triad[22,23] and patients with systemic autoimmune diseases have increased amounts of BLyS in serum or synovial fluid[23].

Mutagenicity among B cells

This discussion led to the third mechanism: B cell mutagenicity. Early B cell developmental events in the bone marrow operate to generate the B cell antigen receptor (BCR). Subsequent events in the periphery allow for further diversification of the BCR—during the course of somatic hypermutation of immunoglobulin genes in the germinal centers of lymphoid follicles of lymph node and spleen—under the influence of T cell help. The three processes are peculiar to the molecular genetics of the adaptive immune system: variable-diversity-joining (V(D)J) recombination, isotype switching and somatic hypermutation. Recombination and somatic mutation, and possibly isotype switching, require double-strand breakage and rejoining of DNA. This provides for economy of gene usage but confers a special risk as a consequence of translocation of oncogenes to the immunoglobulin loci (chromosome 14q32)[24–26]. Under conditions of somatic hypermutation in germinal centers (described as a high-risk zone for lymphomagenesis), potentially pathological chromosomal translocations of oncogenes can occur in more mature B cells concurrent with, or after the occurrence of, V(D)J recombination and class switching. The paradigms include Bcl-2 that is switched from chromosome 18q21.3 to the immunoglobulin locus on chromosome 14, whereby there is encoded an "apoptosis product" that interferes with programmed cell death, as found in most follicular B cell lymphomas, and c-Myc that is switched from chromosome 8q24.3, whereby there is encoded a transcriptional activator of genes required for cell growth, as found in Burkitt lymphomas. Somatic hypermutation in germinal centers occasionally affects nonimmunoglobulin genes such as the Fas receptor, whose inactivation in the germ line results in a severe autoimmune syndrome. Thus, although these somatic genetic events were discussed mainly in relation to lymphomagenesis, their relevance to autoimmunity is obvious (Martin Dyer, Leicester, UK; Tak Mak, Toronto, Canada; Ming-Qing Du, London, UK; Klaus Rajewsky, Cologne, Germany).

Sjögren's syndrome

Among all autoimmune diseases Sjögren's syndrome best illustrates the autoimmunity-lymphoproliferation-lymphoma sequence. The Sjögren's syndrome–associated lymphoproliferation ranges from essentially benign through MALT-type lesions to frankly displastic lymphomas[27]. A notable histological feature that is also seen in various other autoimmune pathologies, particularly autoimmune thyroiditis, is the lymphoid follicle-like structures with germinal centers that simulate the architecture of peripheral lymphoid nodes (**Fig. 1**). The presumed chronic antigen drive in Sjögren's syndrome, virus or autoantigen, is uncertain. EBV has long been under suspicion (but

never convicted) as an instigator of Sjögren's syndrome. Data from the conference cited by Salvatore De Vita (Udine, Italy) suggested that an immune response to the Fc of IgG (the rheumatoid factor autoantigen) may be the crucial event in progression from an inflammatory to a lymphoproliferative disorder. Thus Sjögren's syndrome–associated MALT lymphomas express a very limited repertoire of V_H gene segments. Among the cases examined, half used a V_H1-69 gene segment and many V_H genes were similar in their third complementary-determining regions of antibody paratopes. The implication is that a single antigen is selecting responsive B cells for proliferation and transformation[28]. Finally, restricted V_H gene sequences expressed by many salivary gland lymphoma cells suggest rheumatoid factor activity (David Bahler, Salt Lake City, USA).

Some questions answered

The discussions provided some answers to the questions posed at the beginning of the conference, and which are listed in the opening paragraph of this Commentary.

(i) Associations between autoimmune disease and lymphoma are indeed real, but data are firm for only a few autoimmune diseases and further population-based research is required. Generally, the autoimmune disease precedes the lymphoma. An association does not seem to exist for a number of autoimmune diseases that tend to appear in earlier life, such as autoimmune liver diseases, myasthenia gravis type 1 diabetes and multiple sclerosis. Thus age itself could contribute to the risk of autoimmune-related lymphoma.

(ii) Sustained antigen drive, implicated in lymphomagenesis, can be provided by infectious agents, such as *H. pylori* and HCV, acting independently of autoimmune expression; exogenous antigens that induce autoimmune responses by molecular mimicry; autoantigens, such as the lymphoma associated with Sjögren's syndrome (Fc of IgG); or thyroiditis (thyroid antigens).

(iii) The autoimmune disease–associated lymphomas are overwhelmingly of the B cell type, the major exceptions being the intestinal T cell lymphomas that arise in long-standing celiac disease and in association with infection with the retrovirus HTLV-1.

(iv) Unraveling the associations will have clinical value in allowing for better disease classification, more rational treatment and even prevention of lymphoma either by eradication of persistent infectious agents (*H. pylori* or HCV) or prevention of infection by vaccination. In the future, data derived from gene microarrays done with lymphocytes from blood or biopsied tissue may be able to predict susceptibility to lymphoma in individuals with long-standing autoimmune disease[29].

(v) Fundamental insights will be gained by further study of the coexistence of autoimmunity and lymphoma in humans and mouse models by providing opportunities to dissect the genetic and environmental factors that confer risk for the autoimmunity-lymphoma combination. The clear common elements are abnormal persistence (or inefficient disposal) of lymphocytes due to defective apoptosis, antigen-driven sustained proliferation of lymphocytes with a particular specificity due to exogenous or endogenous antigen and high-risk genetic strategies. These strategies involve the use of double-stranded DNA breaks to achieve diversification of antigen recognition by B cells in germinal centers, thus, for example, facilitating chromosomal translocation of genes for anti-apoptotic molecules into critical immunoglobulin loci.

Catastrophes usually depend upon a combination of errors. In the case of autoimmunity and lymphoma, there is a convergence of multiple genetic traits of both germ line and random somatic genetic origin, together with prolonged antigenic stimulation. There are undoubtedly additional initiating events, such as molecular mimicry occurring on the genetic setting of defective self-tolerance. It is now time to dissect the multiple interdependent and independent components of the autoimmunity-lymphoma association with the goal of adding to our understanding of both processes.

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