Splenic marginal zone lymphoma-like features in API2-MALT1 transgenic mice that are exposed to antigenic stimulation

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Recently, we described a transgenic mouse model to analyze the effect of the API2-MALT1 fusion-protein in vivo. Our results showed that the expression of API2-MALT1 is not sufficient to induce the development of lymphoma masses. Here, we demonstrate that immunization with Freund’s complete adjuvant led to the loss of compartmentalization of the splenic white pulp in API2-MALT1 transgenic mice, resulting in a splenic marginal zone lymphoma-like lymphoid hyperplasia of a peculiar B-cell subset that disappeared as soon as the antigenic stimulation faded away. These data indicate an effect of API2-MALT1 expression on the normal immune response.

Key words: API2-MALT1, MALT lymphoma, immune response.

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Extranodal marginal zone lymphoma or mucosa-associated lymphoid tissue (MALT) lymphoma is listed as a separate disease entity in the World Health Organization (WHO) classification of lymphoid tumors, accounting for 7.6% of all non-Hodgkin’s lymphomas. Among the genetic defects reported in MALT lymphoma, the translocation t(11;18)(q21;q21) is the most common structural abnormality with a frequency ranging from 10% to 45% (mainly lymphomas with gastric or pulmonary localization) depending on the study. The t(11;18)(q21;q21) leads to the generation of a fusion protein comprising the three BIR (baculovirus inhibitor of apoptosis protein repeat) domains present in the N-terminus of the API2 protein and a variable part of the MALT1 protein, containing the caspase p20-like domain. The chimeric protein API2-MALT1 effectively activates nuclear factor κB (NF-κB) in vitro, a transcription factor for a number of survival-related genes. The presence of t(11;18)(q21;q21) correlates with apparent lack of further genetic instability or chromosomal imbalances. In view of this specific association between t(11;18)(q21;q21) and MALT lymphoma, we developed a transgenic mouse model to analyze in vivo the effect of the fusion-protein API2-MALT1 on the development of lymphoid tissue. Transgenic FVB animals were generated via pronuclear injection of an API2-MALT1 fusion construct between exon 7 of API2 and exon 8 of MALT1 driven by the SRα promoter under the control of the immunoglobulin heavy-chain enhancer (Eμ). It was shown that expression of API2-MALT1 alone is not sufficient to induce the development of lymphoma masses within 50 weeks. Nevertheless, it triggered the specific expansion of the splenic marginal zone (Figure 1), affected B-cell maturation in the bone marrow and increased polyubiquitination of the NF-κB essential modulator (NEMO) or IKKγ and, thus, NF-κB activation. Here, we describe the morphologic, immunophenotypic and molecular features of API2-MALT1 transgenic mice after antigenic stimulation.

Design and Methods

The absence of spontaneous lymphoma development in API2-MALT1 transgenic mice suggests that other pathogenic mechanisms, acting synergistically with the API2-MALT1 fusion protein, are essential for the development and growth of a lymphoma. In this respect and considering the association of MALT lymphoma with (auto)antigenic stimulation, 40-week old transgenic mice (n=10), as well as their equally old wild-type littermates (n=10), were exposed to a strong antigenic stimulus, by means of intraperitoneal injection of a single bolus (200 μL) of Freund’s complete adjuvant (Sigma-Aldrich, St. Louis, USA). Antigen-exposed mice were sacrificed 5 weeks later, when the immune response to Freund’s adjuvant is known to be fully developed, as well as 6, 9 and 12 weeks after exposure to antigenic stimulation. Macroscopic findings as well as microscopic features of the spleen, lymph nodes, bone marrow, thymus, gut, lung, liver and salivary glands were analyzed. Hematoxylin and eosin-stained sections (4
µm) of formalin- or B5-fixed, paraffin-embedded biopsies were examined carefully for the presence of histopathological lesions. Serial paraffin sections of the spleen were immunostained for the B-cell marker CD20 (goat polyclonal anti-mouse antibody [Santa Cruz Biotechnology, Santa Cruz, USA]) and the T-cell marker CD3 (rabbit polyclonal anti-mouse antibody [Sigma-Aldrich, St Louis, USA]), whereas frozen sections of the spleen were stained for IgM (rabbit polyclonal anti-mouse antibody [Southernbiotech, Alabama, USA]), IgD (rabbit polyclonal anti-mouse antibody [Southernbiotech, Alabama, USA]) and CD5 (rabbit polyclonal anti-mouse antibody [Pharmingen, San Diego, USA]). According to a previously published protocol,11,12 polymerase chain reaction (PCR) analysis of the CDR3 region of the immunoglobulin heavy-chain gene was performed on selected specimens to analyze the clonal nature of any observed B-cell proliferation.

Results and Discussion

No macro- nor microscopic differences were noted in any of the organs examined after antigenic stimulation with Freund’s adjuvant, except for the spleen. In this secondary lymphoid organ, a striking morphologic difference was found between the white pulp of transgenic and wild-type mice (Figures 2 and 3): wild-type mice preserved a normal distribution of red and white pulp with intact compartmentalization of the follicle mantle into a lymphocytic corona (composed of IgM+/IgD+ B-cells) and a marginal zone (composed of IgM+/IgD− B cells). In contrast, the spleen of transgenic mice was characterized by a very prominent white pulp, composed of very large B-cell areas and poorly developed T-cell areas, and an almost absence of the red pulp. Moreover, compartmentalization of the follicle mantle into a marginal zone and lymphocytic corona complete-

Figure 1. Spleen morphology of API2-MALT1 transgenic mice and wild-type littermates at the age of 20 weeks. Transgenic mice show a reduction of red pulp with an increase of white pulp due to an expanded marginal zone (A) compared with their wild-type littermates (B).

Figure 2. Three weeks after immunization with Freund’s complete adjuvant, the compartmentalization of the follicle mantle had totally disappeared in API2-MALT1 transgenic mice (A), while the mantle zone of the B-follicles in wild-type mice preserved its compartmentalisation into a marginal zone and a lymphocytic corona (B).
ly disappeared. The mantle zone was almost entirely populated by small B-lymphocytes and clusters of these cells could also be seen in the red pulp, sometimes invading the sinuses. Further immunophenotypic characterization showed that these small B-lymphocytes lacked CD5 expression, and were marked by strong surface IgM and IgD expression. These findings indicate that the presence of the API2-MALT1 fusion protein, in combination with strong antigenic stimulation, favors the expansion of a peculiar B-cell population. More importantly, this combination of events results in a disrupted architecture of the splenic white pulp with morphologic and immunophenotypic features that strongly resemble a splenic marginal zone lymphoma as defined by the WHO classification for human lymphoid neoplasms’ and by the Bethesda group for mice lymphoid neoplasms. In humans, this type of non-Hodgkin’s lymphoma is a rare disorder, accounting for less than 1% of lymphoid tumors, and is considered by the WHO classification as a separate disease entity. Although distinct from MALT lymphomas, it is postulated that splenic marginal zone lymphomas also probably arise from a B-cell subset homing in the marginal zone. It is a B-cell neoplasm comprising small lymphocytes which surround (and sometimes replace) germinal centers, efface the follicle mantle and merge with a peripheral marginal zone-like area which includes some transformed blasts. The neoplastic B cells are typically CD5-negative but strongly express surface IgM and IgD, having the identical immunophenotype to that of the expanded B-cell population in the API2-MALT1 transgenic mice after administration of Freund’s adjuvant. Despite significant differences in the architecture of the normal marginal zone between mice and humans, the morphology and immunophenotype of splenic marginal zone lymphoma in mice parallel those of human splenic marginal zone lymphoma remarkably; however, for a reason not yet known, the incidence of splenic marginal zone lymphomas is much higher in some strains of mice (e.g. NZB and NFS.x strains) than in humans. In FVB mice, which were chosen as the background strain for our animal model, splenic marginal zone lymphomas and lymphoproliferative disorders in general are uncommon features, further emphasizing the importance of the observed lymphoid hyperplasia in the splenic white pulp of the antigen-exposed, API2-MALT1 transgenic mice.

PCR analysis of the CDR3 region of the immunoglobulin heavy-chain gene was performed on splenic biopsies, to analyze the clonal nature of the observed B-cell proliferation. Despite clear-cut morphologic and immunophenotypic features indicative of a splenic marginal zone lymphoma, the splenic B-cell proliferation in the antigen-exposed, API2-MALT1 transgenic mice was found to be oligoclonal (4 of 10 cases analyzed; 40%) or polyclonal (6 of 10 cases analyzed; 60%), thereby suggesting the presence of a (premalignant?) lymphoid hyperplasia rather than a malignant lymphomatous
process. Due to this absence of monoclonality, it is more appropriate to describe the observed loss of the immunoaortic architectural pattern in the spleen of the transgenic mice as marginal zone lymphoma-like features rather than define them as genuine splenic marginal zone lymphomas. However, it must be noted that in daily clinical practice, the diagnosis of lymphoma is still primarily based on morphologic and immunohistochecmic analyses, and that PCR can only provide additional arguments in favor of a histologically diagnosed lymphoid malignancy if monoclonality can be demonstrated (the absence of monoclonality does not exclude a lymphoma).

At present, it is unclear why marginal zone lymphoma-like features appear in API2-MALT1 transgenic mice after immunization, since human splenic marginal zone lymphoma is not related to t(11;18)(q21;q21). Differences between the mouse and human immune systems may be responsible for the unexpected appearance of API2-MALT1-induced lymphoma-like features in the splenic marginal zone rather than other locations. It is also of interest to note that the t(11;18)(q21;q21) has been demonstrated in a human marginal zone lymphoma located in the spleen, although it occurred in splenic dissemination of a gastric MALT lymphoma and not a primary splenic marginal zone lymphoma.5 However, in none of the analyzed antigen-exposed transgenic mice was there evidence for the presence of extranodal location(s) of a marginal zone lymphoma. Moreover, as immunization with Freund’s adjuvant did not induce extranodal loci of inflammation,10 the effect of the API2-MALT1 fusion-protein on B-cell aggregates outside the spleen and lymph nodes could not be investigated.

Interestingly, the morphologic changes in the API2-MALT1 transgenic mice gradually faded away and disappeared by 12 weeks after administration of Freund’s adjuvant. Therefore, we assume that the elimination of the antigenic trigger is sufficient to completely restore the normal splenic architecture with intact compartmentalization of the white pulp. At first sight, this phenomenon may appear very similar to the disappearance of gastric MALT lymphomas after therapy to eradicate Helicobacter pylori.11-20 However, regression of gastric MALT lymphomas usually occurs in monoclonal MALT lymphomas that do not carry the t(11;18)(q21;q21). Nevertheless, the reversible, oligo- to polyclonal marginal zone hyperplasia in antigen-exposed, API2-MALT1 transgenic mice indicates an effect of API2-MALT1 expression on the normal immune response.

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