T-bet-positive and IRTA1-positive monocytoid B cells differ from marginal zone B cells and epithelial-associated B cells in their antigen profile and topographical distribution

Korinna Jöhrens
Yoshihiko Shimizu
Ioannis Anagnostopoulos
Stefan Schiffmann
Enrico Tiacci
Brunangelo Falini
Harald Stein

Background and Objectives. It remains controversial whether splenic and nodal marginal zone B cells, monocytoid -, and epithelial-associated B cells represent separate B-cell subsets or just variants of the same population. To clarify this issue we studied the antigen profile and topographical distribution of these cell types.

Design and Methods. We studied samples of toxoplasmic lymphadenopathy, lymph nodes with a developed marginal zone, and hyperplastic palatine tonsils. Deparaffinized sections were subjected to antigen-retrieval pre-treatment then incubated with appropriate antibodies. The bound antibodies were made visible using the alkaline phosphatase anti-alkaline phosphatase method with FastRed as the chromogen.

Results. We found that i) nodal marginal zone B cells have a similar immunophenotype and topographical distribution to their splenic counterparts, ii) monocytoid B cells differ in their antigen profile (presence of T-bet, IRTA1, CD75, CD45RA, absence of BCL-2 , CD21, CD27) from splenic and nodal marginal zone B cells and more closely resemble epithelial-associated B cells (presence of IRTA-1, CD45RA, partial expression of T-bet, CD75, absence of CD21, CD27). Monocytoid B cells were mostly not found in nodal marginal zones when a marginal zone was developed, but were seen in areas adjacent to marginal zones and occasionally in germinal centers.

Interpretations and Conclusions. Collectively, our results indicate that monocytoid B cells represent a distinct differentiated B-cell subset, and provide a solid basis for isolation and functional investigations of the cell types studied, and for revising the hitherto inadequate classification of nodal marginal zone lymphomas.

Key words: T-bet, IRTA1, B cells, marginal zone, monocytoid.

Haematologica 2005; 90:1070-1077

From the Institute for Pathology, Charité, Campus Benjamin Franklin, Medical University Berlin, Germany (KJ, IA, SS, HS); Department of Pathology, Saitama, Medical School, Saitama, Japan (YS); Institute of Hematology, University of Perugia, Perugia, Italy ET, BF).

Correspondence:
Harald Stein M.D., Institute for Pathology Charité, Campus Benjamin Franklin, Medical University Berlin, Hindenburgdamm 30, D-12200 Berlin, Germany.
E-mail: harald.stein@charite.de

Marginal zone B cells of the spleen represent an established B-cell subset, with specific functions, demonstrated in rodent models.[1] These cells have been shown to be anatomically closely related to specialized macrophages, dendritic and endothelial cells. They represent a first line of defense against blood-borne pathogens.[2] In humans, B cells with similar cytological features and localization are also found outside the spleen,[3] namely in inborn mucosa associated lymphoid tissue/MALT (i.e. Peyer's patches of the ileum) and in mesenteric lymph nodes (nodal) marginal zone B cells. The precise relationship between the splenic and nodal marginal zone B cells is still far from clear. Moreover, another conspicuous B-cell population has been identified which appears to resemble marginal zone B cells in terms of localization. This population is most consistently induced by *Toxoplasma gondii* infection[4] and usually seen in cervical lymph nodes.[5] Three different names have been assigned to this B-cell population: *monocytoid B cells*,[6] *sinus B cells*[7] or *nodal marginal zone B cells*.[8] The different names bear witness to the controversy regarding their relationship to marginal zone B cells. One view favors that marginal zone B cells and monocytoid B cells represent closely related variants of the same B-cell subset.[8-13] This point of view is based on the claim that splenic marginal zone B cells and monocytoid B cells share a similar topographical distribution, a similar immunophenotype (expression of IgM in the absence of IgD) and a similar immunoglobulin (Ig) heavy chain variable (VH) gene mutation pattern. Some authors[8-13] have therefore subsumed the monocytoid B cells under the term *nodal marginal zone B cells*. On the other hand, studies by our group[6,14] support the view that the monocytoid B cells are distinct from marginal zone B cells and represent a separate B-cell subset, justifying the term *monocytoid B cells*. This view relies on the observation that while splenic marginal zones contain moderate numbers of T

cells, monocytoid B-cell proliferates are completely devoid of such cells but contain numerous neutrophils.[7] The immunophenotype of marginal zone B cells also differs significantly from that of monocytoid B cells: the marginal zone B cells are positive for IgM and BCL-2[15] and negative for the KiB3-epitope of CD45RA, whereas monocytoid B cells express strongly and consistently the KiB3-epitope of CD45RA but little or no IgM and no BCL-2.[6] In addition, in our single cell studies, the Ig VH mutation patterns differed in both cell populations in that most splenic marginal zone B cells contained IgH mutations but monocytoid B cells did not. These data suggest that most marginal zone B cells originate from mutated post-germinal center B cells and the majority of monocytoid B cells from pre-germinal center B cells.[6] In order to provide a solid basis for a classification of neoplasms derived from these cell subsets, more distinguishing proteins are required. IgH mutations are not suitable for this purpose because their demonstration is too labor-intensive and thus too expensive and, more importantly, they are not specific enough for subtyping lymphomas originating from post-germinal center B cells.

Finally, another B-cell subset seems to have a close relationship to the marginal zone B-cell zones, the epithelial-associated B cells.[16,17] This B-cell population is located underneath and within tonsil epithelia and in the dome epithelium of Peyer's patches. As recently described,[18] these epithelial-associated B cells have a memory B-cell phenotype with expression of CD27 and BCL-2 antigens. Furthermore, the topographical distribution and the morphology of the epithelial-associated B cells[19] support this notion.

The data cited above[6,8,20] have not been regarded as sufficiently convincing to conclude that marginal zone B-cells and monocytoid B cells represent different B cell populations. It is also not clear whether epithelial-associated B cells are closely related to marginal zone B cells or whether they, too, represent another B-cell population. It has also been argued that the described differences between monocytoid B cells and marginal zone B cells might be due to the different environments in which the cells occur.[7] A clear definition of marginal zone B cells, monocytoid B cells and epithelial-associated B cells is essential for at least for two reasons: (i) to provide a tool for their distinction in tissue sections and cell suspensions and, thus, a means by which these cell subsets can be isolated from cell suspensions for further studies, e.g. gene expression profiling and functional studies; and (ii) to precisely identify the lymphoma categories which may derive from these B-cell populations. Thus far the so-called nodal marginal zone lymphomas appear to be a quite heterogeneous category presumably consisting of a mixture of neoplasms originating from nodal marginal zone B cells and from monocytoid B cells. For the purpose of clarifying these issues we extended our previous investigations by searching for further differences in the antigen expression profiles and topographical distiribution of marginal zone B cells, monocytoid B cells and epithelial-associated B cells.

One of the novel molecules investigated is IRTA1,[21] a member of the immunoglobulin-like receptor family, present in resting mature B cells.[22] IRTA1 is selectively and consistently expressed by monocytoid B cells and by epithelial-associated B cells.[18] The second novel molecule is the T-box transcription factor, T-bet, which is essential for Th1 lineage commitment.[23,24] We found T-bet helpful in discriminating between marginal zone B cells and monocytoid B cells as it is consistently present in monocytoid B cells. In addition to IRTA1 and T-bet, we found three further antigens (CD21, CD27 and CD75) that are differentially expressed in marginal zone B cells and monocytoid B cells. CD75 is a cell surface sialyltransferase and has been characterized as a B-cell surface molecule expressed predominantly on mature and activated B lymphocytes, while CD27 is highly expressed in memory B-cells. Finally CD21 is a complement (C3d) and Epstein-Barr virus receptor and mainly expressed in follicular dendritic cells and in splenic marginal zone B cells.

Design and Methods

Tissue specimens

We studied seven spleen samples and nine mesenteric lymph node samples from 16 different patients as well as cervical lymph node specimens with features of toxoplasmic lymphadenopathy from nine patients, drawn from the archives of the Institute of Pathology of the Charité, Campus Benjamin Franklin of the University Medical School, Berlin, Germany. In addition, we included six hyperplastic palatine tonsils in the study in order to compare the various B-cell populations with those present in the tonsil, especially those located in close vicinity to and within crypt epithelium (epithelial-associated B cells).

Methods

Four micrometer thick deparaffinized sections were subjected to antigen-retrieval pre-treatment employing high pressure cooking using either citrate (pH 6.0) or EDTA (pH 8.0) buffer. After this pre-treatment, the sections were incubated with the primary antibody for 30 min. In the following only those antibodies which proved to contribute to the definition of the characteristic antigen profile of the cell populations under investigation are mentioned. These

antibodies include anti-CD3 (polyclonal), anti-CD20 (clone L26), anti-CD21 (clone 1F8), anti-CD27 (clone 137B4), anti-CD45RA (clone KiB3), anti-MUM1/IRF4 (clone MUM1p), anti-IRTA1 (clone M-IRTA1), anti-CD75 (clone LN1), and anti-CD23 (clone 1B12). Anti-CD45RA (KiB3) was a gift from the laboratory of Dr. R. Parwaresch Kiel, Germany. IRF4/MUM1 and IRTA1 antibodies were generated in the laboratory of Dr. B. Falini, Perugia, Italy; their characteristics have been previously described.[25] The anti-CD23 antibody was purchased from Novocastra laboratories, Newcastle, UK, the anti-CD27 from Coulter-Beckman, San Diego, USA, the anti-CD75 from Serotec, Oxford, UK, while the remaining antibodies were obtained from DAKOCytomation, Glostrup, Denmark. Finally the monoclonal antibody against the T-bet (clone 39D) was purchased from Santa Cruz, California, USA. Bound antibodies were made visible using the alkaline phosphatase anti-alkaline phosphatase (APAAP) method employing FastRed as the chromogen.

Results

To better define the phenotype of marginal zone B cells and monocytoid B cells we screened a large number of antibodies using specimens rich in these cells (spleen and Piringer's lymphadenitis respectively). The molecules which proved most significant in helping to elucidate the features of the cell populations investigated were T-bet, IRTA1, CD21, CD27, KiB3 epitope of CD45RA and CD75 in addition to previously described antigens (IgM, BCL2).[6] The reactivity of the antibodies directed against the mentioned molecules with various normal tissues is described below and summarized in Table 1.

Splenic marginal zone B cells

In all seven spleen samples investigated, a similar immunostaining pattern of the cells in marginal zones (Figure 1A) was noted with expression of BCL-2 (Figure 1B), CD21 (Figure 1C) and CD27 (Figure 1E), while CD45RA (Figure 1D) and CD75 (Figure 1F) were almost completely absent. Expression of IRTA1 (surface membrane positivity) and T-bet (nuclear positivity) was not detected on most splenic marginal zone B cells with only a few cells being weakly positive (Figure 1G and H).

Nodal marginal zone B cells

In all nine mesenteric lymph node samples, the marginal zone B cells (Figure 2A) expressed BCL-2 (Figure 2B) and weakly, but specifically, CD27 (Figure 2E), i.e. to the same intensity as on the splenic marginal zone B cells. In contrast, expression of IRTA1 and T-bet (Figure 2G and H) was not detectable. A very small proportion of these cells also expressed CD45RA and CD75 (Figure 1D and F), while the reactivity for CD21 ranged from negative to faintly positive (Figure 2C).

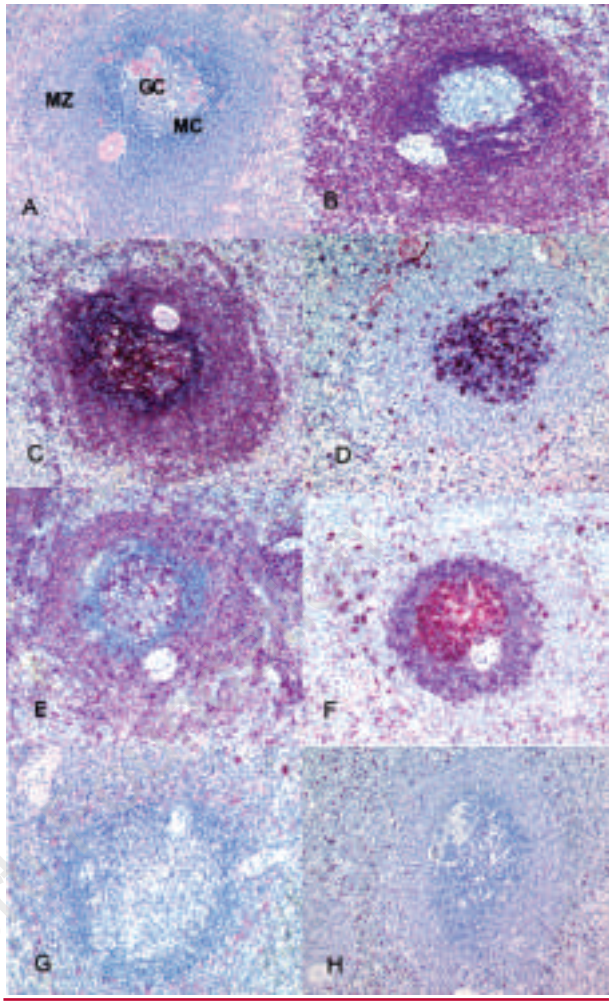

Figure 1. Spleen: histology and immunostains for BCL-2, CD21, KiB3, CD27, CD75, IRTA1 and T-bet. (A) Giemsa staining: typical germinal center surrounded by mantle and marginal zones. (B) BCL-2: the marginal zone B cells are intensively stained. (C) CD21: the marginal zone B cells are strongly labeled. (D) KiB3: the cells in the marginal zone are negative. (E) CD27: the marginal zone B cells express CD27 less intensively than do the T cells. (F) CD75: the marginal zone B cells are predominantly negative. (G) IRTA1: a few cells of the marginal zone are weakly stained. (H) T-bet: the marginal zone is negative. Only a T-cell subpopulation is positive.

Monocytoid B cells

In all nine cases with toxoplasmic lymphadenopathy (Figure 3A), the monocytoid B cells which exhibited a *common type* morphology[26] showed, irrespectively of their distribution, strong expression of T-bet (Figure 3H), IRTA1 (Figure 3G), CD45RA (Figure 3D) and CD75 (Figure 3F) while BCL-2 (Figure 3B), CD27 (Figure 3E) and CD21 (Figure 3C) were not detectable. In the search for lymphoid tissue in

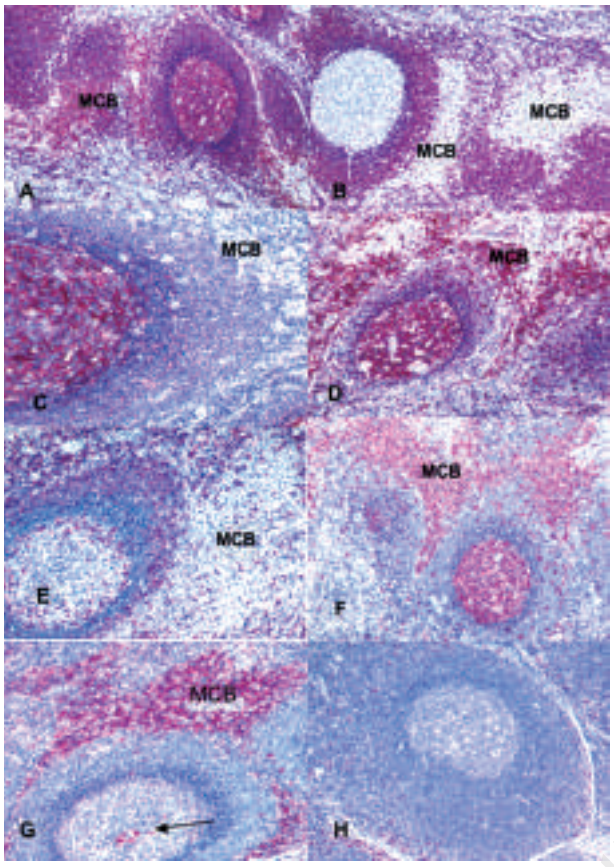

Figure 2. CD20, BCL-2, CD21, KiB3, CD27, CD75 and IRTA1 immunostains in a mesenteric lymph node containing monocytoid B-cell sheets and a prominent marginal zone. (A) CD20: the monocytoid B cells (MCB) exhibit a slightly different red tone from that of the marginal zone B cells. (B) BCL-2: the marginal zone B cells are positive while the monocytoid B cells are negative. (C) CD21: the marginal zone B cells show a weak positivity, the monocytoid B cells are negative. (D) KiB3: the monocytoid B cells are strongly positive whereas only a few marginal zone B cells are stained. (E) CD27: the marginal zone B cells are positive for CD27, but the monocytoid B cells are negative. (F) CD75: the marginal zone B cells are negative whereas the monocytoid B cells are strongly positive. (G) IRTA1: only the monocytoid B cells lying in sheets and within the germinal center (arrow) are positive. (H) T-bet: the marginal zone B cells are negative.

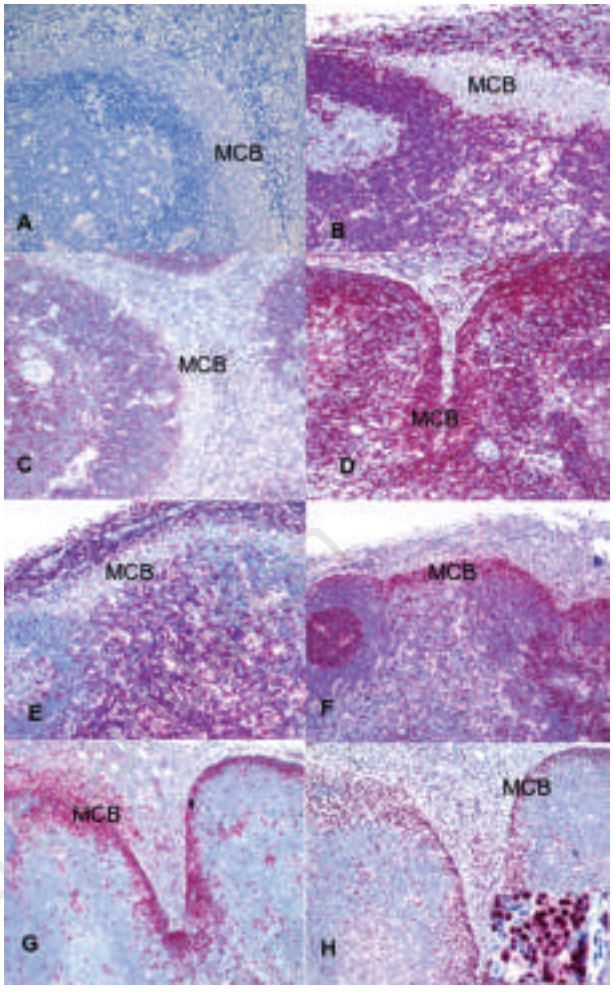

Figure 3. Morphology and immunostains for BCL-2, CD21, KiB3, CD27, CD75, IRTA1 and T-bet of monocytoid B cells in Piringer's lymphadenopathy. (A) Giemsa staining: sheet of monocytoid B cells adjacent to a secondary follicle. (B) BCL-2: monocytoid B cells are negative. (C) CD21: note the follicular dendritic cells, which are absent in the area of the monocytoid B cells. (D) KiB3: the monocytoid B cells show strong positivity. (E) CD27: the monocytoid B cells are negative. Note the labeling of rare intermingled T cells. (F) CD75: the monocytoid B cells and the germinal center cells are intensely stained. (G) IRTA1: the monocytoid B cells are labeled. (H) T-bet: the monocytoid B cells (in a higher magnification in the insert picture), and a T-cell subpopulation are positive.

which marginal zone B cells and monocytoid B cells are simultaneously present we found three mesenteric lymph nodes with foci of sheets of monocytoid-like cells attached to but clearly separated from the marginal zones (Figure 2A-G). The separation was surprisingly sharp since there were almost no IRTA1-positive or T-bet-positive cells detectable in the marginal zone. However, single or a few IRTA1-positive and T-bet-positive cells were encountered in several germinal centers of toxoplasmic lymphadenopathy (Figure 3G and H) and the mesenteric lymph nodes with monocytoid B cells (Figure 2G). The germinal centers of not activated lymphoid tissues devoid of sheets of monocytoid B cells did not contain IRTA1-positive or T-bet-positive cells in any case. The monocytoid B cells present in the mesenteric lymph nodes proved to have the same antigen expression profile as that of the monocytoid B cells of toxoplasmic lymphadenopathy (Figure 2B-G and 3B-H). The cellular composition of these cell sheets was also similar to the monocytoid B cell zones in *Toxoplasma gondii* lymphadenopathy (absence of T cells and presence of neutrophils). The monocytoid B cell zones of the cervical and mesenteric lymph nodes were either located near and/or around marginal and/or intermediary sinuses. Because of these completely congruent characteristics of the *Toxoplasma gondii*-induced monocytoid B cells and the B cells of the zones attached to marginal zones adjacent to sinuses in the

mesenteric lymph nodes, we concluded that the latter cells also represent true monocytoid B cells. We are of the opinion that the same is true for the IRTA1-positive and T-bet-positive B cells in the germinal centers.

Epithelial-associated B cells

In accordance with previous findings, immunostaining for IRTA1 (Figure 4G) molecule led to the labeling of many cells in the crypt epithelium of tonsils (Figure 4A). Immunostaining for T-bet (Figure 4H) showed that a minor proportion of the epithelial-associated B cells cells is also positive for T-bet. These cells proved to be negative for the MUM1/IRF4 molecule, confirming that they are B cells without any differentiation towards plasma cells. Further immunostaining showed strong reactivity of the epithelial-associated B cells for the KiB3-epitope of CD45RA (Figure 4D), similar to monocytoid B cells. However, unlike monocytoid B cells, these cells expressed BCL-2 (Figure 4B) while CD75 (Figure 4F) was negative in the majority of the IRTA1-positive cells; CD21 (Figure 4C) and CD27 (Figure 4E) molecules were detectable on a minority of these cells.

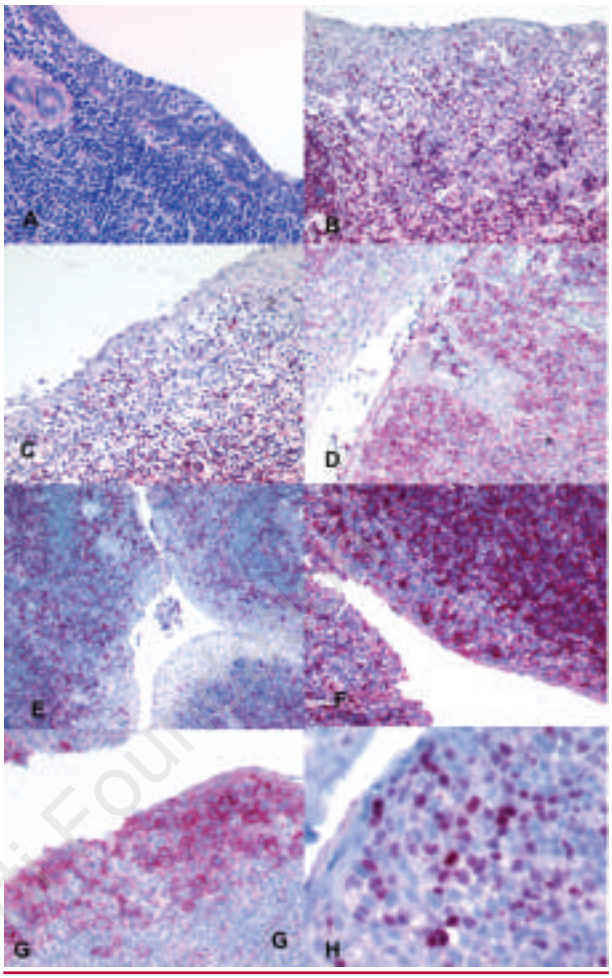

Figure 4. Epithelial-associated B cells: morphology and immunostains for BCL-2, CD21, KiB3, CD27, CD75, IRTA1 and T-bet. (A) Giemsa staining: detail of a tonsillar crypt with lymphoid cells between squamous epithelial cells. (B) BCL-2: the epithelial-associated B cells are positive. (C) CD21:only a few epithelial-associated B cells are positive. (D) KiB3: the epithelial-associated B cells are strongly labeled. (E) CD27: the majority of the epithelial-associated B cells are negative. (F) CD75: Only a subpopulation of epithelial-associated B cells is positive. (G) IRTA1: the epithelial-associated B cells are selectively stained. (H) T-bet: positive T-bet expression in a few nuclei of the epithelial-associated B cells.

Discussion

B cells that home to within and adjacent to marginal zones, sinuses and epithelia are likely to be those B lymphocytes which are the first to encounter antigen in the lymphoid tissues.[27] Although these B cells share morphologic similarities there are still many controversies regarding their relationships and functions. One of the major questions to be answered concerns the relationship between marginal zone B cells and monocytoid B cells: one view regards monocytoid B cells as nodal counterparts of marginal zone B cells[8-13] while the other considers that monocytoid B cells represent a distinct B-cell population unrelated to marginal zone B cells.[6] The present study provides strong arguments in favor of the latter concept. Our extended comparison of the immunophenotype of the splenic marginal zone B cells with that of *Toxoplasma gondii*-induced monocytoid B cells revealed five new additional differences in antigen expression: monocytoid B cells were positive for IRTA1, T-bet and CD75 and negative for CD21 and CD27 whereas the splenic marginal zone B cells showed an opposite reactivity.

The next question to be answer was whether the previously and presently found differences in antigen expression may be due to different environments. To clarify this issue we looked for lymphoid tissue samples in which monocytoid B cells and marginal zone B cells occur side by side. Since monocytoid B cells were never identified in spleens and also not in the seven spleens included in the present study, we concentrated our search on lymph nodes. In line with present knowledge, well-developed spleen-like marginal zones were only found in some mesenteric lymph nodes. The comparison of the antigen profile of the cells present in the marginal zones of the spleen and the mesenteric lymph nodes showed that they share a similar but not identical immunophenotype. Both cell populations had in common the expression of IgM at a high level and IgD at low level, CD27 at a low level and BCL-2 at a high level. The main difference concerned expression of the complement receptor for C3d (CD21), which proved to be high in splenic marginal zone B cells but absent or

Table 1. Summary of the immunophenotypes that discriminate between marginal zone B cells, monocytoid B cells and epithelial-associated B cells.

Cell population	*T-bet*	*IRTA1*	*CD21*	*CD27*	*BCL2*	*CD45RA*	*CD75*
MZB cells*							
spleen	–	-/single+	+	+	+	–	-/single+
mesenteric LN#	–	-	-/+	+	+	-/single+	-/single+
Monocytoid B cells							
toxoplasmic LN	+	+	–	–	–	+	+
mesenteric LN	+	+	–	–	–	+	+
EAB cells**							
tonsil	-/+	+	-/single+	-/single+	+	+	-/+

**MZB cells: marginal zone B cells; ** EAB cells: epithelial-associated B cells, #LN: lymph nodes.*

low in nodal marginal zone B cells. This finding is consistent with the special function of splenic marginal zone B cells which is their capacity to bind thymus-independent (TI-2) antigens via complement receptors and to initiate an immediate immune response to these antigens usually expressed by encapsulated bacteria.[3] Minor differences between marginal zone B cells of the spleen and mesenteric lymph nodes concerned CD45RA and IRTA1, whereas no differences regarding CD75 were observed (Table 1). Since the similarities predominated over the differences, the marginal zone B cells of the mesenteric lymph nodes can be regarded as related to splenic marginal zone B cells. The expression of CD27 is consistent with the view that both marginal zone B-cell populations belong to the memory pool of the B-cell lineage.[28-31]

Having established the close relationship between splenic and nodal marginal zone B cells we searched for the presence of monocytoid B cells in mesenteric lymph nodes and succeeded, on three occasions, in finding lymph nodes with large spleen-like marginal zones. In these cases we found zones of B cells with monocytoid features which were in the direct vicinity of, but clearly separated from, the marginal zones. These B cells proved to have an immunophenotype and cellular background identical to that of *Toxoplasma gondii*-induced monocytoid B-cell proliferates, indicating that: (i) the cells of these extra-marginal zones represent true monocytoid B cells; and (ii) the antigen expression program of monocytoid B cells is not influenced by the location of the lymph node and/or presence or absence of a spleen-like marginal zone. More importantly, the side by side occurrence of monocytoid B cells and marginal zone B cells allowed a direct comparison of the immunophenotypic characteristics of both cell populations. This comparison confirmed the above mentioned differences in antigen expression and underscored the concept that marginal zone B cells and monocytoid B cells represent different B-cell subpopulations. An intriguing additional finding was that monocytoid B cells were not detectable within the marginal zones, indicating that monocytoid B cells and marginal zone B cells do not compete in their homing attitudes. The zones to which monocytoid B cells home proved to be associated with sinuses, in line with our earlier findings which had led us to designate monocytoid B cells as *B-cell sinus cells*.[5] However, single or a few IRTA1-positive and T-bet positive B cells were regularly encountered in several germinal centers of lymphoid tissues with sheets of monocytoid B cells but not in lymphoid tissues devoid of such sheets of cells. This finding indicates that monocytoid B cells are able to migrate to germinal centers. It is tempting to speculate that this observation might be able to explain why most monocytoid B cells do not harbor IgH hypermutations but a few do. It is conceivable that the monocytoid B cells are, in general, derived from naïve B cells but acquire IgH hypermutations when they migrate to germinal centers. Since obviously only a few monocytoid B cells find their way to germinal centers, most monocytoid B cells remain with a non-mutated IgH status.

The antigen expression program of monocytoid B cells is characterized, among other features, by the presence of two newly described molecules T-bet and IRTA1.[22,32] T-bet is regarded as a key transcription factor promoting TH1 differentiation by inducing interferon-γ production.[33] Recent *in vitro* data have provided evidence that T-bet can be up-regulated in B cells by application of oligodeoxynucleotides containing a CpG motif.[34] Experimental studies in mice additionally demonstrated that T-bet influences Ig class switching, which can occur in response to T-cell-independent,[35] but not T-cell-dependent, stimuli. Whether the latter observations in mice can serve as a model for human monocytoid B cells remains a matter of speculation. Based on our earlier findings that: (i) monocytoid B cells contain solely IgG

mRNA[14] in the absence of any Ig protein and ii) the extreme rarity of T cells within monocytoid B-cell proliferates, we are tempted to speculate that T-bet may actually cause Ig class switch recombination in monocytoid B cells in a T-cell-independent setting. Thus the monocytoid B cells might represent a specialized B-cell subset able to deal with pathogens in a T-cell-independent manner analogous to that of splenic marginal zone B cells. The mechanism does, however, appear to be different as T-bet up-regulation is only found in monocytoid B cells. The role of IRTA1 expression in monocytoid B cells is unclear. IRTA1 has been established to be a surface B-cell receptor related to Fc-receptors, whose functions regarding involvement in immune responses, intercellular communication and cell migration are still largely unknown.[21]

Regarding the expression of IRTA1, epithelial-associated B cells in tonsils were found to have a closer relationship to the monocytoid B cells than to the marginal zone B cells.[18] In the present study, we confirm and further expand previous findings, showing similarities but also differences between monocytoid B cells and epithelial-associated B cells. In fact, both monocytoid B- and epithelial-associated B-cell subsets usually show positivity for IRTA1 and CD45RA. Regarding expression of T-bet and CD75, only a variable proportion of the epithelial-associated cells are positive, whereas both molecules are strongly expressed in monocytoid B cells. Furthermore, while all epithelial-associated B cells express BCL-2 and occasionally CD21 and CD27, the monocytoid B cells are consistently negative for all three antigens. Notably, both epithelial-associated B cells and monocytoid B cells show the same IgH mutation pattern (a mixture of mutated and unmutated cells).[18,6] A possible explanation for the phenotypic and genotypic similarities between these two B-cell subsets may be related to their interaction with epithelial and/or sinus lining cells in areas where antigen is known to arrive and be presented. Chemokines might also well be involved in the homing mechanism of these cells.

Taken together, the results of our study show that: (i) marginal zone B cells of spleen and lymph node are closely related although they might differ in their functions i.e. in the reactivity with TI-2 antigens; (ii) irrespective of some similiarities the marginal zone B cells and monocytoid B cells differ distinctly in their antigen expression profile. The splenic marginal zone B cells and the monocytoid B cells may share the ability to react to pathogens in a T-cell-independent way; (iii) the epithelial-associated B cells appear to be more closely related to monocytoid B cells than to marginal zone B cells.

HS had the idea, designed the study and drafted the manuscript; KJ and IA supervised the data collection, interpretated the data, wrote and revised the manuscript; YS, SS selected the cases analyzed, collected the data and assisted in their interpretation; ET, BF provided important technical and intellectual contributions to the manuscript. The final version was approved by all co-authors.This work was supported by grants from the Deutsche Krebshilfe, Deutsche Forschungsgesellschaft and the Associazione Italiana per la Ricerca sul Cancro.

The authors are particularly indebted to Constanze Cieluch and Sandra Meier for their expert technical assistance. The authors would also like to thank Sarah Coupland for help and advice.

Manuscript received February 17, 2005. Accepted June 28, 2005.

References

1. Martin F, Kearney JF. Marginal-zone B cells. Nat Rev Immunol 2002;2:323-35.
2. Balazs M, Martin F, Zhou T, Kearney J. Blood dendritic cells interact with splenic marginal zone B cells to initiate T-independent immune responses. Immunity 2002;17:341-52.
3. Zandvoort A, Timens W. The dual function of the splenic marginal zone: essential for initiation of anti-TI-2 responses but also vital in the general first-line defense against blood-borne antigens. Clin Exp Immunol 2002; 130: 4-11.
4. Dorfman RF, Remington JS. Value of lymph-node biopsy in the diagnosis of acute acquired toxoplasmosis. N Engl J Med 1973;289:878-81.
5. Ahmad M, Iqbal J, Mansoor A, Khan AH. Toxoplasmic lymphadenitis-a clinicopathological study. J Pak Med Assoc 1991;41:303-5.
6. Stein K, Hummel M, Korbjuhn P, Foss HD, Anagnostopoulos I, Marafioti T, et al. Monocytoid B cells are distinct from splenic marginal zone cells and commonly derive from unmutated naive B cells and less frequently from postgerminal center B cells by polyclonal transformation. Blood 1999;94:2800-8.
7. Stein H, Lennert K, Mason DY, Liangru S, Ziegler A. Immature sinus histiocytes. Their identification as a novel B-cell population. Am J Pathol 1984; 117: 44-52.
8. Tierens A, Delabie J, Michiels L, Vandenberghe P, Wolf-Peeters C. Marginal-zone B cells in the human lymph node and spleen show somatic hypermutations and display clonal expansion. Blood 1999;93:226-34.
9. Wolf-Peeters C, Pittaluga S, Dierlamm J, Wlodarska I, Van Den BH. Marginal zone B-cell lymphomas including mucosa-associated lymphoid tissue type lymphoma (MALT), monocytoid B-cell lymphoma and splenic marginal zone cell lymphoma and their relation to the reactive marginal zone. Leuk Lymphoma 1997;26:467-78.
10. van den Oord JJ, Wolf-Peeters C, De Vos R, Desmet VJ. Immature sinus histiocytosis. Light- and electron-microscopic features, immunologic phenotype, and relationship with marginal zone lymphocytes. Am J Pathol 1985; 118:266-77.
11. Fend F, Kraus-Huonder B, Muller-Hermelink HK, Feller AC. Monocytoid B-cell lymphoma: its relationship to and possible cellular origin from marginal zone cells. Hum Pathol 1993; 24: 336-9.
12. Nizze H, Cogliatti SB, von Schilling C, Feller AC, Lennert K. Monocytoid B-cell lymphoma: morphological variants and relationship to low-grade B-cell lymphoma of the mucosa-associated lymphoid tissue. Histopathology 1991; 18:403-14.
13. Piris MA, Rivas C, Morente M, et al. Monocytoid B-cell lymphoma, a tumour related to the marginal zone. Histopathology 1988;12:383-92.
14. Stein K, Hummel M, Korbjuhn P, Foss HD, Anagnostopoulos I, Marafioti T,m et al. Monocytic B-cells represent a new cell population that is mainly recruited from unmutated polyconal naive B-cells. Verh Dtsch Ges Pathol 2000;84:151-2.
15. Meda BA, Frost M, Newell J, Bohling SD, Huebner-Chan DR, Perkins SL, et al. BCL-2 is consistently expressed in hyperplastic marginal zones of the spleen, abdominal lymph nodes, and ileal lymphoid tissue. Am J Surg Pathol 2003;27:888-94.
16. Dono M, Burgio VL, Tacchetti C, Favre A, Augliera A, Zupo S, et al. Subepithelial B cells in the human palatine tonsil. I. Morphologic, cytochemical and phenotypic characterization. Eur J

Immunol 1996;26:2035-42.
17. Dono M, Zupo S, Augliera A, Burgio VL, Massara R, Melagrana A, et al. Subepithelial B cells in the human palatine tonsil. II. Functional characterization. Eur J Immunol 1996;26:2043-9.
18. Falini B, Tiacci E, Pucciarini A, Bigerna B, Kurth J, Hatzivassiliou G, et al. Expression of the IRTA1 receptor identifies intra-epithelial and sub-epithelial marginal zone B cells of the mucosa-associated lymphoid tissue (MALT). Blood 2003;102:3684-92.
19. Morente M, Piris MA, Orradre JL, Rivas C, Villuendas R. Human tonsil intraepithelial B cells: a marginal zone-related subpopulation. J Clin Pathol 1992;45:668-72.
20. Tierens A, Delabie J, Wolf-Peeters C. Monocytoid B cells. Blood 2000; 96: 1612-4.
21. Hatzivassiliou G, Miller I, Takizawa J, Palanisamy N, Rao PH, Iida S, et al. IRTA1 and IRTA2, novel immunoglobulin superfamily receptors expressed in B cells and involved in chromosome 1q21 abnormalities in B cell malignancy. Immunity 2001;14:277-89.
22. Miller I, Hatzivassiliou G, Cattoretti G, Mendelsohn C, Dalla-Favera R. IRTAs: a new family of immunoglobulinlike receptors differentially expressed in B cells. Blood 2002; 99: 2662-9.
23. Lighvani AA, Frucht DM, Jankovic D, Yamane H, Aliberti J, Hissong BD, et al. T-bet is rapidly induced by interferon-γ in lymphoid and myeloid cells. Proc Natl Acad Sci USA 2001; 98:-15137-42.
24. Szabo SJ, Sullivan BM, Stemmann, Satoskar AR, Sleckman BP, Glimcher LH. Distinct effects of T-bet in TH1 lineage commitment and IFN-γ production in CD4 and CD8 T cells. Science 2002;295:338-42.
25. Falini B, Fizzotti M, Pucciarini A, Bigerna B, Marafioti T, Gambacorta M, et al. A monoclonal antibody (MUM1p) detects expression of the MUM1/IRF4 protein in a subset of germinal center B cells, plasma cells, and activated T cells. Blood 2000;95:2084-92.
26. Plank L, Hansmann ML, Fischer R. The cytological spectrum of the monocytoid B-cell reaction: recognition of its large cell type. Histopathology 1993; 23:425-31.
27. Morse HC 3rd, Kearney JF, Isaacson PG, Carroll M, Fredrickson TN, Jaffe ES. Cells of the marginal zone-origins, function and neoplasia. Leuk Res 2001; 25:169-78.
28. Agematsu K, Nagumo H, Yang FC, Nakazawa T, Fukushima K, Ito S, et al. B cell subpopulations separated by CD27 and crucial collaboration of CD27+ B cells and helper T cells in immunoglobulin production. Eur J Immunol 1997;27:2073-9.
29. Agematsu K, Hokibara S, Nagumo H, Komiyama A. CD27: a memory B-cell marker. Immunol Today 2000;21:204-6.
30. Klein U, Rajewsky K, Kuppers R. Human immunoglobulin (Ig)M+IgD+ peripheral blood B cells expressing the CD27 cell surface antigen carry somatically mutated variable region genes: CD27 as a general marker for somatically mutated (memory) B cells. J Exp Med 1998;188:1679-89.
31. Tangye SG, Liu YJ, Aversa G, Phillips JH, de Vries JE. Identification of functional human splenic memory B cells by expression of CD148 and CD27. J Exp Med 1998;188:1691-703.
32. Davis RS, Wang YH, Kubagawa H, Cooper MD. Identification of a family of Fc receptor homologs with preferential B cell expression. Proc Natl Acad Sci USA 2001;98:9772-7.
33. Szabo SJ, Sullivan BM, Stemmann C, Satoskar AR, Sleckman BP, Glimcher LH. Distinct effects of T-bet in TH1 lineage commitment and IFN-gamma production in CD4 and CD8 T cells. Science 2002;295:338-42.
34. Rifkin IR, Marshak-Rothstein A. T-bet: the Toll-bridge to class-switch recombination? Nat Immunol 2003;4:650-2.
35. Gerth AJ, Lin L, Peng SL. T-bet regulates T-independent IgG2a class switching. Int Immunol. 2003;15:937-44.

Yellowstone National Park, USA ROBIN FOÀ