Extrafollicular proliferation of B cells in the absence of follicular hyperplasia: a distinct reaction pattern in lymph nodes correlated with primary or recall type responses

A Brighenti, M Andrulis,[1] E Geissinger,[1] S Roth,[1] H K Müller-Hermelink[1] & T Rüdiger[1]
Istituto di Anatomia Patologica, Università degli Studi di Verona, Verona, Italy, and [1]Institute of Pathology, University of Würzburg, Würzburg, Germany

Brighenti A, Andrulis M, Geissinger E, Roth S, Müller-Hermelink H K & Rüdiger T
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Aims: Extrafollicular activation of B cells is rarely observed in human lymph nodes. The aim of this study was to extensively analyse the expression of surface molecules and transcription factors in four such cases, comparing them with follicular B cells and medullary cord plasma cells.

Methods and results: Various combinations of B-cell-related surface markers and transcription factors were studied by triple immunofluorescence. While in the germinal centre, reactive immunoglobulin production occurred exclusively in non-proliferating cells, in extrafollicular activation proliferation of B cells and immunoglobulin production coexisted. In two of these cases proliferating cells were mainly IgG+CD27+, i.e. derived from class-switched postgerminal centre memory B cells. Some of these cells expressed CD30. In the other two cases, immunoglobulin-forming cells were non-class-switched IgM+CD27− B cells, representing a primary expansion of naive B cells.

Conclusions: Extrafollicular B-cell activation is the morphological correlate of rapid B-cell responses that do not involve the germinal centres. It is pathogenetically heterogeneous, comprising primary responses that occur prior to, or independent of, germinal centre reaction or memory cell activation in recall responses.

Keywords: B-cell development, extrafollicular, primary immune response

Introduction

In reactive lymph nodes, B cells, T cells and histiocytes may proliferate and differentiate independently in their respective compartments. B-cell responses usually occur in the form of follicular hyperplasia. In germinal centres, activated B cells proliferate and differentiate, finally becoming memory cells and plasma cells. The passage through the germinal centre is accompanied by changes in gene expression;[1] thus in relation to the germinal centre reaction distinct B-cell subsets can be defined by characteristic transcription factors and surface molecules (Figure 1). In the early stages of development, the transcription factors PAX-5[2] and Pu.1[3] regulate the

differentiation of B cells. Within the germinal centre, bcl-6 and CD10 are up-regulated in proliferating (Ki67+) B cells.[4] Frequently, immunoglobulin heavy chain classes are switched from the μ and δ chains, which are characteristically expressed in naive B cells, to other classes, mainly γ. Following germinal centre exit, bcl-6 and CD10 disappear while CD27 is up-regulated. In humans, therefore, CD27 represents a marker of B cells that have undergone a germinal centre reaction.[5–8]

The development of antibody-forming plasma cells is also tightly regulated:[9,10] B cells committed to a plasma cell fate express molecules such as BLIMP-1,[11–13] IRF-4[14,15] and later CD138, transcription factors and surface proteins that are not expressed in memory cells. BLIMP-1, when inducing plasmacytic differentiation, represses proliferation, germinal centre function and B-cell receptor signalling.[16]

Address for correspondence: Dr Thomas Rüdiger, Department of Pathology, Josef-Schneider-Str. 2, D-97080 Würzburg, Germany. e-mail: thomas.ruediger@mail.uni-wuerzburg.de
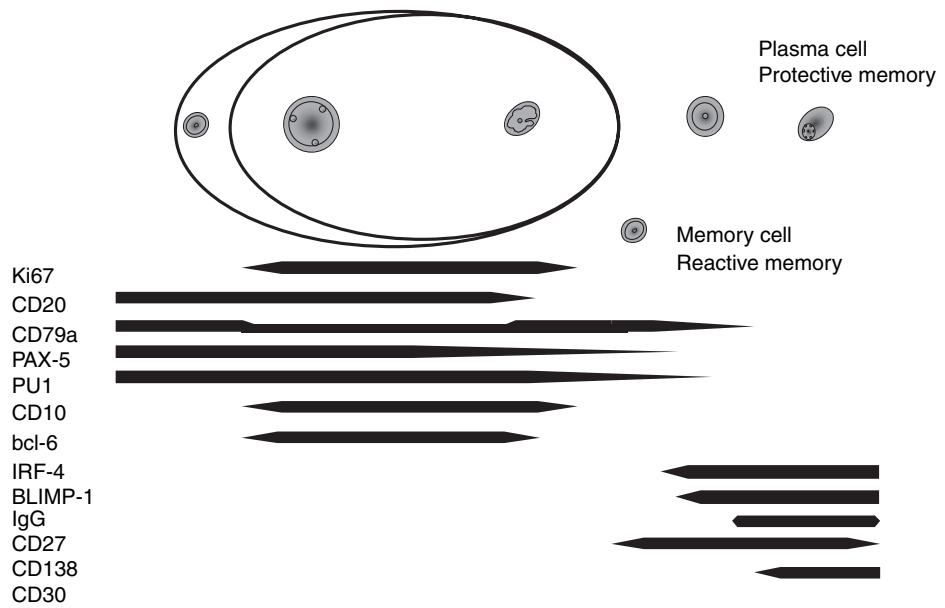
Figure 1. Sequential expression of surface markers and transcription factors in the germinal centre reaction.

In the paracortex only scattered B cells occur, among them blasts with dendritic morphology.[17] Expansions of the paracortical pulp are caused by activated T cells reacting to (auto)immune alterations or viral infections. Prominent B-cell proliferation in the paracortex has not been studied in human lymph nodes. In this study we present four cases with prominent extrafollicular B-cell activation occurring in the paracortical pulp without significant follicular involvement. Characterizing the B blasts immunophenotypically by double and triple immunofluorescence, these could be related to distinct stages of B-cell development in response to antigen as described in animal models.

Material and methods

We collected paraffin-embedded tissue from four lymph nodes showing prominent extrafollicular activation of B cells with resting or only slightly activated B-cell follicles. These were compared with four cases of usual follicular hyperplasia, two of which also showed prominent medullary cord plasmocytosis. In addition, two cases of usual paracortical T-cell activation, including one case of infectious mononucleosis, were studied. All cases were stained conventionally (haematoxylin and eosin, periodic acid–Schiff, Giemsa) and immunohistochemically to establish the diagnosis.

To analyse the different B-cell subpopulations, immunofluorescent triple staining was performed using multiple antibody combinations (Table 1). In some cases, primary antibodies from three different

Table 1. Antibody combinations used for the study

CD79a+IRF-4+	CD3, CD20 PU.1, PAX-5 bcl-6, CD10 IgL, CD138 CD30, bcl-2 BLIMP-1 Ki67 bcl-2
CD79a+Ki67+	CD3 IgL, IgM, IgG BLIMP-1
CD79a+ CD27+	IgG, IgM
CD79a+bcl-2+	IgL, PAX-5
CD79a+ IgL+	CD138
CD27+BLIMP-1+	IgM, IgG CD138 IRF-4
CD27+ CD138+	IgL, IgM, IgG
CD138+ Ki67+	IgG, IgM
PAX-5+IRF-4+	CD3, CD20 CD138
IRF-4+BLIMP-1+	Ki67 IgM, IgG

Triple stains were made by combining the antibodies in the left column with each of the antibodies in the right column.

species were used and were detected with fluorescent-labelled secondary antibodies against the immuno-globulin of the respective species. Antibodies from different species were incubated simultaneously. If two antibodies from the same species had to be employed, one of them was detected conventionally with secondary antibodies against the respective species' immunoglobulin. To prevent cross-reactions, the other primary antibody was FITC-labelled and the signal was amplified with rabbit- or goat-derived secondary antibodies against FITC, followed by their detection with tertiary species-specific Cy2-labelled antibodies derived from donkey. Similarly, biotin-labelled anti-bodies were used to avoid cross-reactions. Blocking was achieved with mouse serum (Dianova, Hamburg Germany; 1 : 20). Alternatively, primary antibodies were directly labelled with fluorescent dyes using the Zenon labelling kit (Molecular Probes, Leiden, the Netherlands) and could be visualized directly. Dilutions and sources of all primary and secondary

Table 2. Sources and dilutions of primary antibodies

Antibody	Clone	Source	Dilution	Conjugation	Detection
Ki67	MM1	Novocastra	1 : 30	ZA488	Donkey α-mouse CY5
CD3	Polyclonal (rabbit)	Dako	1 : 2		Donkey α-rabbit CY3
CD20	L26	Dako	1 : 1000		Donkey α-mouse CY3
CD79a	JCB 117	Dako	1 : 10	FITC	Goat α-FITC
					Donkey α-goat CY2
					Rabbit α-FITC
					Donkey α-rabbit CY2
PU.1	Rabbit	Santa Cruz	1 : 50		Donkey α-rabbit CY3
PAX-5	24	BD	1 : 50		Donkey α-mouse CY3
bcl-6	PG B6p	Dako	1 : 5		Donkey α-mouse CY3
CD10	56C6	Novocastra	1 : 5		Donkey α-mouse CY3
CD27	137B4	Novocastra	1 : 20	Biotin	Streptavidin CY5
IRF-4	Goat	Santa Cruz	1 : 200		Donkey α-goat CY5
IgL	Rabbit	Dako	1 : 10 000		Donkey α-rabbit CY3
IgG	Rabbit	Dako	1 : 10 000		Donkey α-rabbit CY3
IgM	Rabbit	Dako	1 : 10 000		Donkey α-rabbit CY3
CD138	CBL588	Dianova	1 : 50		Donkey α-mouse CY5
CD30	BerH2	Dako	1 : 10		Donkey α-mouse CY3
bcl-2	124	Dako	1 : 40		Donkey α-mouse CY3
BLIMP-1	Rabbit	Provided by A. Avramidou and H.-M. Jäck, Erlangen, Germany	1 : 2000		Donkey α-rabbit CY3

BD, BD Biosciences, San Diego, CA, USA; Dako Cytomation, Hamburg, Germany; Dianova, Hamburg, Germany; Novocastra, Newcastle, UK; Santa Cruz, San Diego, CA, USA; ZA488, Zenon Technology Alexa 488 from Molecular Probes, Leiden, the Netherlands. All secondary antibodies from Dianova, Hamburg, Germany. CD27 and CD79a was conjugated with biotin and FITC by Dianova.

antibodies are given in Table 2. A typical staining protocol was as follows:

Antibody diluent as blocking reagent	15 min
Mouse anti-Ki67 and goat anti-IRF-4	1 h
Donkey anti-mouse CY3 and donkey anti-goat CY5	1 h
Mouse serum	1 h
Mouse anti-CD79a (FITC-labelled)	1 h
Rabbit anti-FITC	1 h
Donkey anti-rabbit CY2	1 h

Between steps, slides were washed three times for 5 min in Tris-buffered saline. After staining, slides were mounted with antifading medium (Fluormount G, Biozol, Eching, Germany) and kept at 4°C in the dark. To detect immunoglobulin (IgL) expression, antibodies against both κ and λ light chains were mixed and detected with the same secondary antibody. The images were evaluated by a confocal laser scanning microscope (Leica TCS SP2; Leica, Bensheim Germany). For each antibody combination, multiple images were taken from different areas of the lymph node: the mantle zone, the germinal centres, the paracortical pulp and the medulla. Populations occurring in the respective compartments were compared with each other.

Detailed clinical data could not be obtained as the disease was self-limiting and no detailed diagnostic procedures were performed.

Results

A summary of the sequential expression of surface markers and transcription factors in germinal centre and extrafollicular responses is given in Figures 1–3.

Figure 2. Sequential expression of surface markers and transcription factors in memory cell activation.
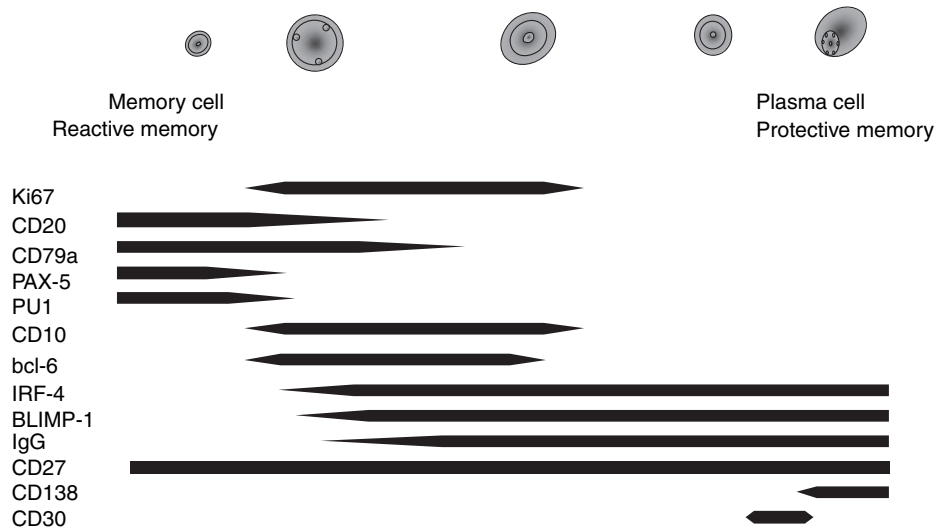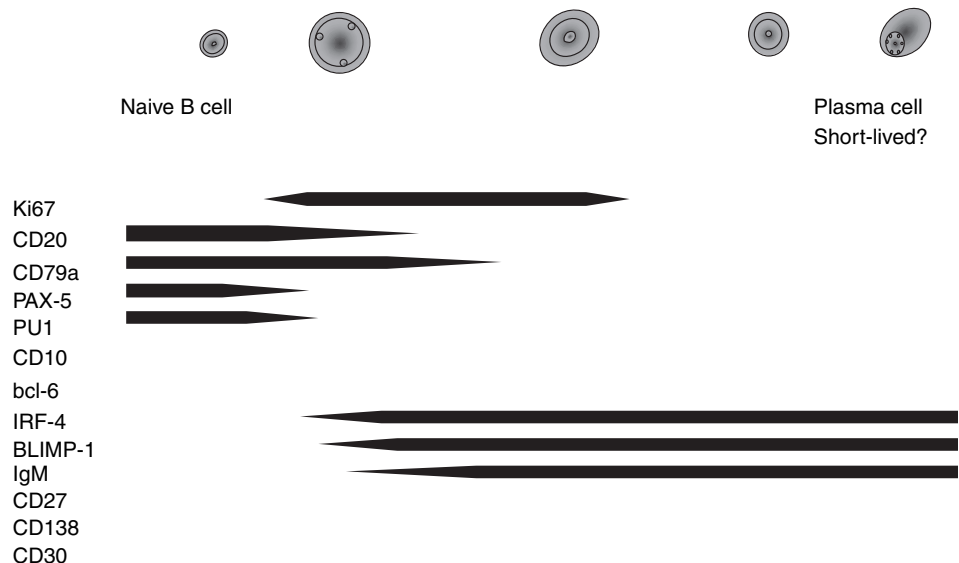

Figure 3. Sequential expression of surface markers and transcription factors in the primary response.
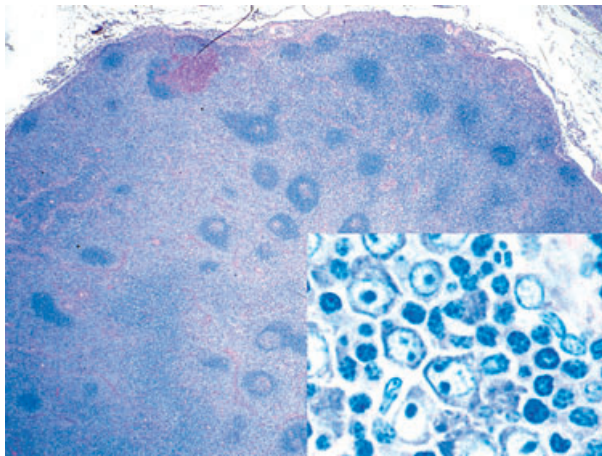
Figure 4. Morphology of extrafollicular B-cell activation (Giemsa). See text for description of the findings.

MORPHOLOGY AND IMMUNOPHENOTYPE OF EXTRAFOLLICULAR BLASTS

Lymph nodes with extrafollicular B-cell activation were enlarged up to 20 mm. Microscopically, their overall structure was preserved with open sinuses. In the cortex, there were few small follicles, some of which contained small germinal centres, but most were primary follicles (Figure 4). The paracortical zones were largely expanded by polymorphous infiltrates ranging from cells resembling centroblasts to immunoblasts to plasmablasts and mature-appearing plasma cells. Within this continuum, morphologically distinct populations could be detected which were also characterized by subsequent changes in their antigen expression (see below). At first, there were small blasts, which had two or three times the diameter of a small lymphocyte. The nuclei had a prominent nuclear membrane and an open chromatin pattern. There were one or two round, sharply demarcated, eccentrically located nucleoli. Some larger cells had nucleoli attached to the nuclear membrane, thus resembling centroblasts. The relatively abundant cytoplasm was moderately basophilic and concentrically located around the nucleus. At the next (second) stage, blasts were greatly enlarged and reached about three times the diameter of those previously described. The nuclei were round or oval with a less prominent nuclear membrane. There was one very prominent centrally located nucleolus, which could be larger than a small lymphocyte. It was intensely stained and sharply demarcated, but often irregular. The cytoplasm was very similar but somewhat less abundant than that described in stage I blasts. Third stage blasts were smaller again, reaching three to four times the diameter of a small lymphocyte. The

nuclear chromatin was open and the nucleolus was smaller, often roundish and more centrally located. The cytoplasm was distinctly basophilic and eccentric, often with a pale-staining Golgi area. These cells resembled immunoblasts. At the fourth stage, the blasts had even smaller nuclei with more basophilic chromatin and multiple, often poorly demarcated eccentric nucleoli which were sometimes present in a spoke-wheel formation. The cytoplasm of these cells was identical to that of mature plasma cells. Towards the perivascular lymphoid sheaths, there was differentiation into morphologically typical plasma cells.

Immunophenotypically, the blasts had a high proliferation rate of 80% or more when labelled with Ki67. In contrast to infectious mononucleosis, they lacked the pan-T-cell markers CD3 and CD5, both of which highlighted a few intermingled small T cells. Using triple stains for Ki67, CD3, and CD79a or immunoglobulin light chains (IgL), respectively, there was a dominant proliferation of CD79a+ and/or IgL+, but not of CD3+ cells.

Stage I blasts expressed both CD20 and PAX-5. Both of these B-cell markers were negative in the later stages of development. Similarly, CD79a and PU.1 were positive in these stage I blasts but only weakly positive in the larger stage II blasts and negative in the later forms. In contrast, IRF-4 was negative at stage I and in mature plasma cells but was expressed in stage II, III and IV blasts. BLIMP-1 expression was similar to IRF-4 but it was also present in plasma cells. Immunoglobulin light chains, which were polytypic, were weakly expressed in stage II blasts and the expression peaked in stage IV blasts and plasma cells. CD138 was weakly expressed in stage III blasts and prominently expressed in stage IV blasts and plasma cells. Interestingly, the expression of CD30 was limited to the large stage II blasts and both earlier and later stages were negative.

PROLIFERATING B CELLS IN THE FOLLICULAR AND EXTRAFOLLICULAR COMPARTMENTS

The peculiarities of extrafollicular B-cell activation may be better appreciated by comparing them with proliferating blasts and Ig-forming cells, respectively, which appear in the well-defined germinal centre reaction.

Proliferating Ki67+ germinal centre B cells expressed Pax-5 and Pu.1, similar to resting B cells in the mantle zone. However, the follicular markers CD10 and bcl-6 were strongly positive, whilst CD20 and CD79a were rather weakly expressed. In the germinal centre, Ki67+ B cells were consistently negative for IRF-4 or BLIMP-1, transcription factors that induce immunoglobulin
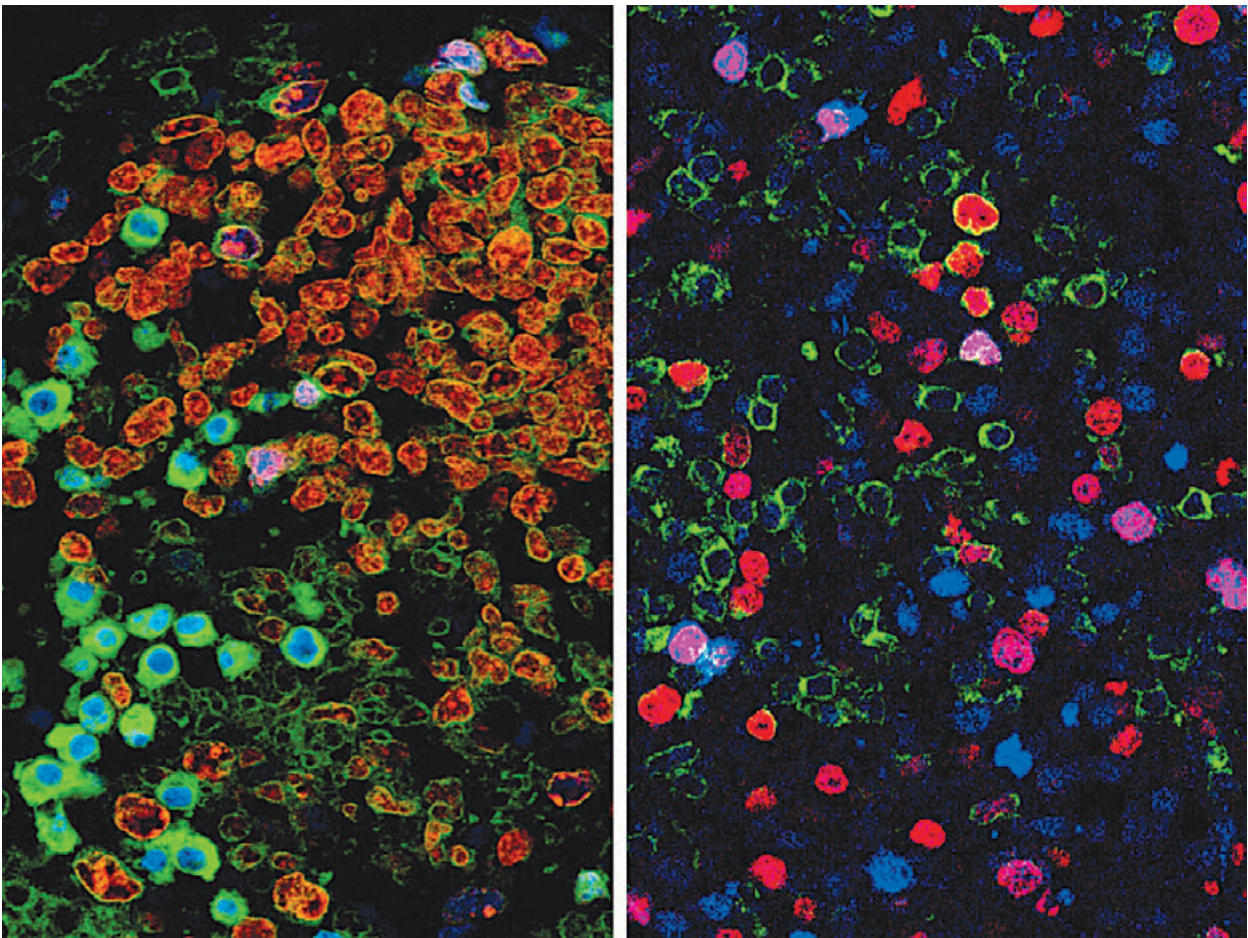
Figure 5. Expression of CD79a (green), Ki67 (red) and IRF-4 (blue) in follicular (left) and extrafollicular (right) activation: while proliferating follicular cells are IRF-4–, coexpression of Ki67 and IRF-4 occurs in extrafollicular B blasts. IRF-4+CD79– cells correspond to immunoglobulin-forming cells with down-regulated CD79a (IgL+CD138+).

secretion. Both transcription factors were detected in a few germinal centre B cells that were non-proliferating and only exceptionally coexpressed CD10 or bcl-6. These cells strongly expressed CD79a and IgL and were located in the periphery of the light zone. Apparently, proliferation ceases with the induction of plasma cell differentiation in germinal centre B cells.

In cases of extrafollicular B-cell activation, in contrast, proliferation coincided with plasma cell differentiation: in the interfollicular area, the proliferating Ki67+ B-cell population usually coexpressed IRF-4 or BLIMP-1 (Figure 5). On the other hand, Pu.1, Pax-5, CD10 and bcl-6, markers observed in proliferating germinal centre B cells, were not observed at all in Ki67+ extrafollicular B cells. In about 50% of proliferating Ki67+ extrafollicular cells, CD79a and ⁄ or CD20 were expressed; their expression levels being considerably higher compared with those in germinal centres. About 50% of BLIMP-1+Ki67+ or IRF-4+Ki67+ cells expressed cytoplasmic IgL. Proliferating cells only rarely coexpressed CD138. This plasma cell-associated marker was seen in a minor Ki67– population.

IMMUNOGLOBULIN-FORMING CELLS IN GERMINAL CENTRE AND EXTRAFOLLICULAR REACTIONS

Regardless of their localization, IgL+ cells in all cases shared the expression of the transcription factors IRF-4 and BLIMP-1. Rarely, IgL– cells were seen that, in addition to CD79a, expressed IRF-4 or BLIMP-1. Both transcription factors were mostly detected simultaneously: in a few CD79a+ cells IRF-4 could be observed in the absence of BLIMP-1, but no BLIMP-1+IRF-4– cells were encountered, apart from mature CD138+ plasma cells. About 50% of BLIMP-1+ or IRF-4+ cells exhibited cytoplasmic IgL. IgL was consistently expressed in mature CD138+ plasma cells. IgL+ cells that
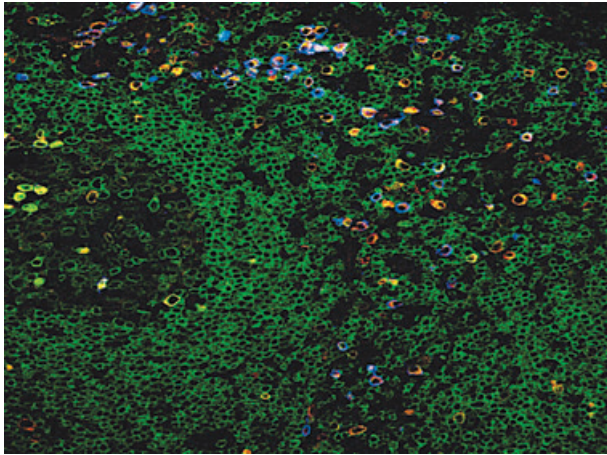
Figure 6. While B cells expressing CD79a (green) and IgL (red) are negative for CD138 (blue) in the follicle, maturation to CD138+ cells occurs in the medullary sinuses.

were negative for CD138 occurred both in follicles, where they were IgG+ or IgM+, and in paracortical zones, where they almost exclusively expressed IgM.

Mature CD138+ plasma cells were variably present in the medullary cords of all cases, but most prominent in two cases of follicular hyperplasia. With the confocal laser scanning microscope, they were easily identifiable by their eccentric cytoplasm containing IgL. These plasma cells represented a non-proliferating Ki67– population. The stronger their IgL and CD138 expression, the weaker was the expression of CD79a, the latter being negative in 50%. Analysing their immunoglobulin (Ig) heavy chains, more than 95% of CD138+ plasma cells produced the γ class, while the μ heavy chain was rarely seen (Figure 6). Regardless of their heavy chain class, the vast majority of CD138+ cells expressed the postfollicular marker CD27.
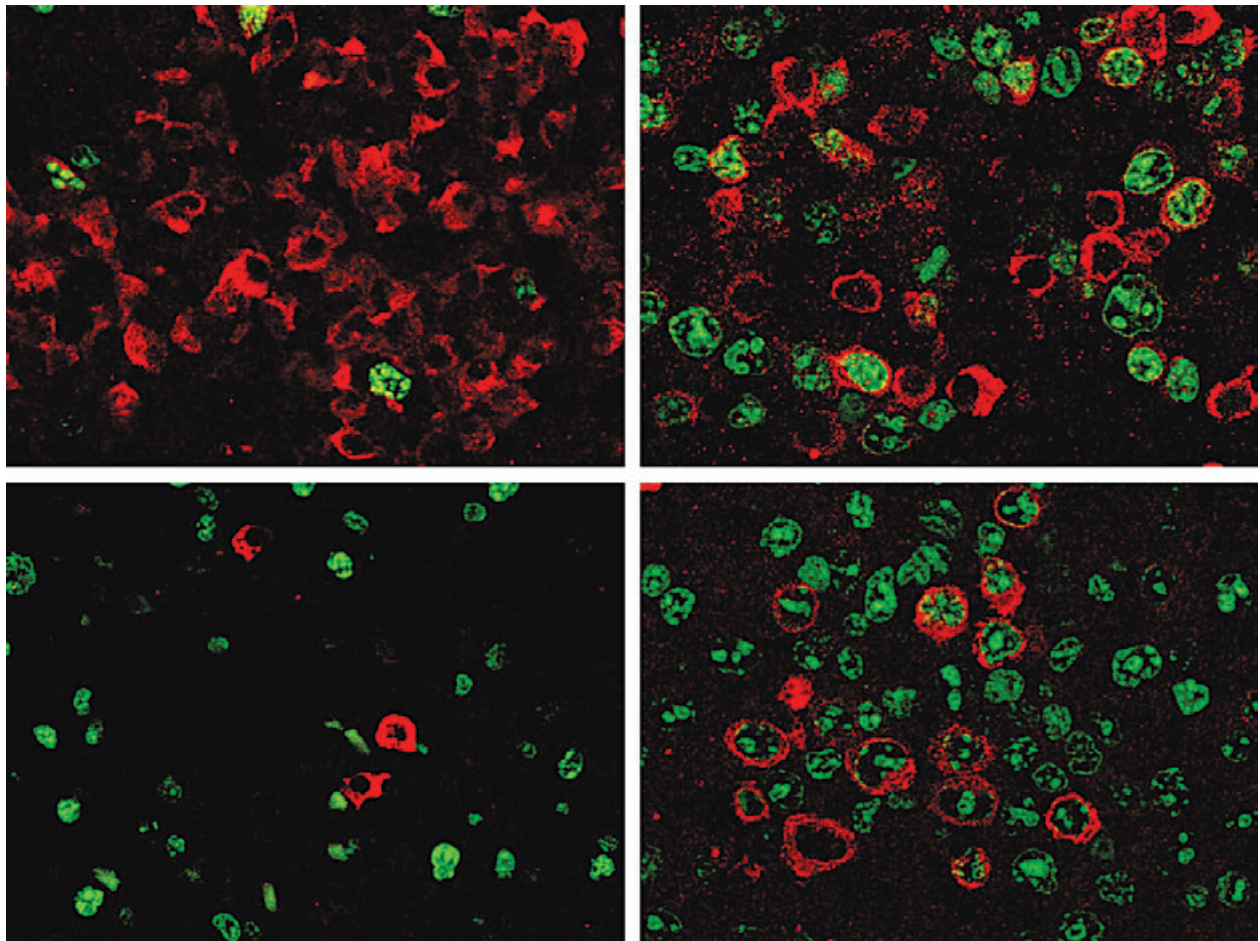

Figure 7. Expression of Ki67 (green) and IgG (red, upper panels) or IgM (red, lower panels) in medullary cords (left) and extrafollicular activation (right). In medullary cords, plasma cells are non-proliferating and mainly express IgG, while in extrafollicular activation proliferating immunoglobulin-forming cells may be IgG or IgM. [Ki67+Ig– cells in the right panel correspond to a CD79a+Ki67+IRF-4+ population, not (yet) expressing immunoglobulin.]
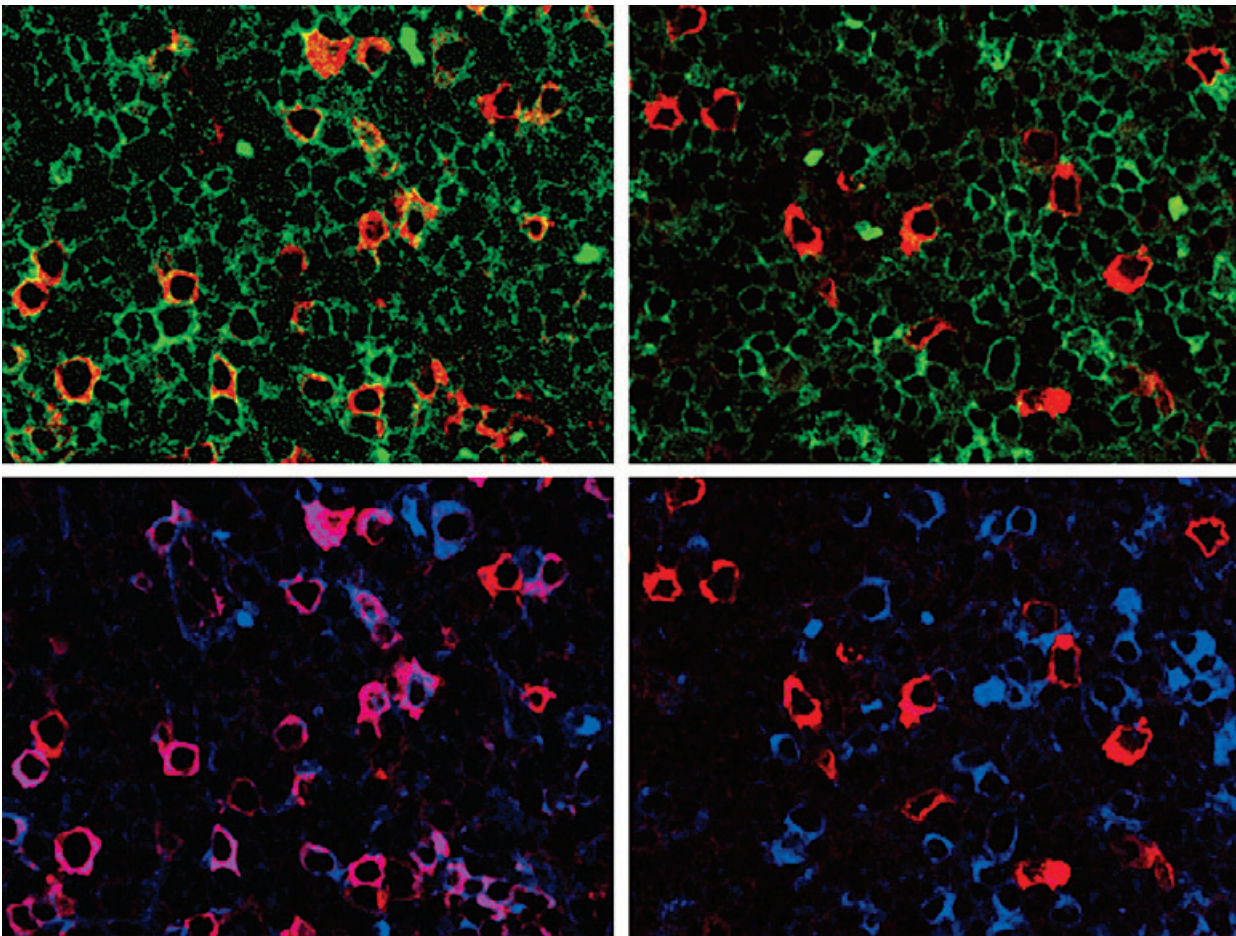
Figure 8. Differential expression of CD27 (green) and CD138 (blue) in cells producing IgG (red, left) and IgM (red, right): while class-switched IgG+ cells express CD27 and CD138, morphologically similar cells expressing IgM are CD27– and do not usually express CD138.

In addition, non-proliferating Ig-forming cells could also be observed in almost all germinal centres. Characteristically, these were few in number and located in the light zones. With their eccentric cytoplasm and nuclear morphology, they resembled plasma cells and mostly expressed the γ and rarely the μ heavy chain. These IgL+ cells were mostly negative for CD20 but strongly positive for CD79a, while CD138 was rarely expressed, thus exhibiting subtle differences compared with mature medullary cord plasma cells (Figure 6).

In contrast to resting plasma cells in medullary cords and in germinal centres, Ig-forming cells in paracortical regions of extrafollicular B-cell activation were proliferating (Figure 7) and comprised a more heterogeneous population (Figure 8).

In two cases, IgL+ cells predominantly expressed the non-class-switched μ heavy chain. The IgM+ population was mainly negative for CD27 and CD138 but CD79a was strongly coexpressed in 90% of these cells. These IgM+ cells made up the major fraction of proliferating cells, being positive for Ki67 in about 75%, while the minor IgG+CD138+ population had a lower Ki67 index.

In the other two cases, the majority of Ig-forming cells produced the (class-switched) γ heavy chain. IgG+ cells also made up the majority of proliferating cells, being Ki67+ in about 75%. Almost all IgG+ cells coexpressed CD138 and they were positive for CD27, an antigen expressed after exiting the germinal centre. The transcription factor and surface marker expression seen in the CD27+ population and resulting plasma cells were similar to mature plasma cells seen in medullary cords.

CD30 expression was noticed in some of the extrafollicular plasmoblasts but was exclusively seen in the IgG+CD27+ population, while it did not occur in IgM+ plasmablasts. These CD30+ blasts were all positive for IRF-4 and variably expressed CD79a, with either low expression or negativity in most of them (Figure 9).
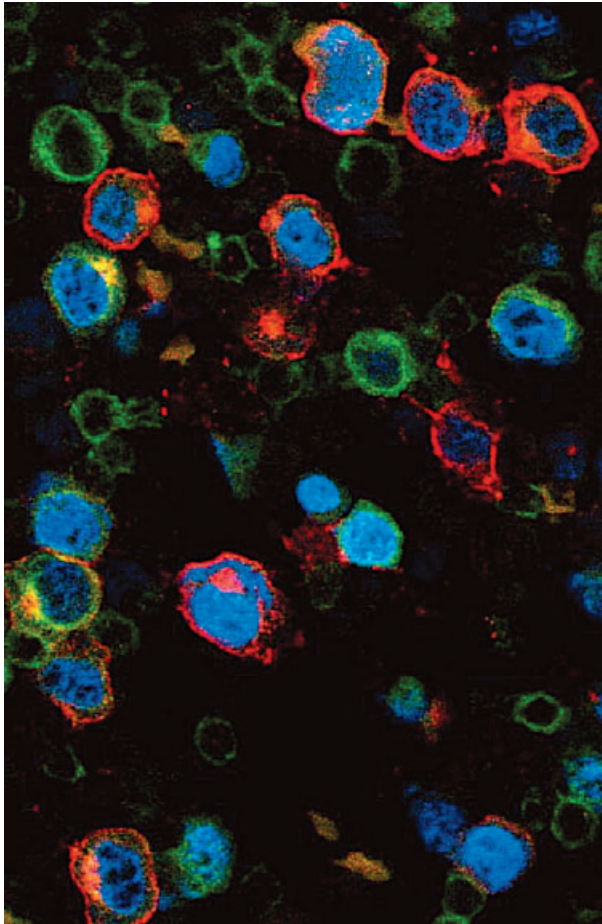
Figure 9. CD30+ (red) extrafollicular plasmablasts were mostly CD79a– (green) and IRF-4+ (blue).

In summary, T cells do not seem to contribute significantly to extrafollicular B-cell activation. The infiltrate consisted of variable proportions of two different populations of Ig-forming cells, one being IgG+CD138+CD79a–CD27+ and the other IgM+CD138–CD79a+CD27–. In contrast to the germinal centre reaction, proliferation and differentiation towards Ig-forming cells occurred simultaneously in the reactive extrafollicular B-cells.

Discussion

We have analysed the immunomorphology of four cases of extrafollicular activation of B cells by double and triple immunofluorescence and compared them with six cases exhibiting prominent T-cell activation and germinal centre reaction. There were clear-cut differences between these well-described processes: in extrafollicular B-cell activation, T cells were not prominently involved and proliferation occurred sim-

ultaneously with differentiation to Ig-forming cells, while both processes occurred subsequent to each other in the germinal centre reaction and its resulting medullary cord plasma cells.

Morphologically, extrafollicular activation of B cells was characterized by an expansion of the paracortex in the absence of follicular hyperplasia. The infiltrate consisted of cells that resembled centroblasts or immunoblasts or had an eccentric basophilic cytoplasm and spoke-wheel formation of their nuclear chromatin, consistent with plasmablastic differentiation. Different stages of maturation were detected side by side. The proliferating Ki67+ blasts produced polytypic cytoplasmic immunoglobulins. Because of their high proliferation index, they differed from Ig-forming cells occurring in medullary cords or in the light zones of secondary follicles, populations that were non-proliferating.

To understand the nature of these immune reactions, we defined the phenotype of the infiltrating B cells by double and triple immunofluorescence and correlated these results with known stages of B-cell development. Based on their expression of immunoglobulin heavy chains, extrafollicular plasmablasts can be subdivided into one non-class-switched population expressing IgM and a class-switched one expressing IgG. These populations could not be distinguished morphologically but differed in their CD27 expression. While the IgM+ population was CD27–, most of the IgG+ plasmablasts were CD27+. CD27 is up-regulated in B cells exclusively after germinal centre activation and can thus be regarded as a specific marker for postgerminal centre B cells in humans.[18–21] This suggests a different derivation of both populations: while the IgG+ population are derived from postgerminal centre B cells, the IgM+ blasts have apparently not undergone germinal centre activation.

Both populations can be correlated to specific stages of immune reactions. Exposure to antigens usually initiates complex events: a rapid, transient primary response peaks after 4 days and low-affinity IgM antibodies are produced. The more powerful secondary response peaks at 8–10 days[22] and involves a germinal centre reaction with somatic hypermutations and an immunoglobulin class switch. It gives rise to long-lived plasma cells, effector cells that produce high-affinity antibodies that are usually class-switched,[23,24] and memory cells. Third, in recall responses, memory cells proliferate and generate effector cells, thus creating a vigorous and robust response[25] which is even more rapid then the primary one.[26] The morphological characteristics of primary and recall responses are

poorly described in humans but have been extensively studied in the extrafollicular compartments of the spleen of genetically engineered mice.[27,28]

In two cases of extrafollicular activation, the immuno-globulin-forming cells were mainly Ki67+IgM+CD27−. Regarding the derivation of the B-cell expansion in these cases, several strands of evidence suggest a mechanism independent of the formation of germinal centres: the extrafollicular proliferation and differentiation has been observed in the absence of follicular hyperplasia. IgM expression alone may rarely occur in a non-class-switched postgerminal centre population,[6,29,30] but their failure to express CD27 also argues against a passage through the germinal centre.[18,19,21] Although the non-proliferating subset of these IgM+CD27− cells resembled plasma cells, they were usually positive for CD79a and always negative for CD138. Apparently, these effector cells do not acquire a mature plasma cell phenotype. Recently, a population of interfollicular proliferating B cells has been described scattered in the paracortical T zones of resting lymph nodes.[17] In contrast to the population described here, these were positive for PAX-5 and mostly negative for IgM, although CD27 was undetectable.

In the other two cases, the prevalent population consisted of proliferating, class-switched postgerminal centre cells (Ki67+IgG+CD27+). This phenotype occurs in B cells that have undergone a successful germinal centre reaction.[18–21] The weaker CD79a was in these cells, the stronger was CD138. Thus, these cells immunophenotypically resemble mature CD79a−IgG+CD138+ medullary cord plasma cells, except for their high proliferation rate. Whereas plasma cells are mitotically quiescent, memory cells are capable of consecutive phases of expansion to generate effector cells when re-stimulated.[26,31] We therefore considered these two specimens as representing a recall response in human lymphoid tissue, where re-stimulated memory B cells proliferate and mature into plasma cells. Sequential expression of surface markers and transcription factors in primary and memory responses are given in Figures 2 and 3.

CD30 was exclusively expressed in a subset of these IgG+CD27+ plasmablasts, but not in IgM+CD27− cells. With the exception of immunoglobulin expression, these CD30+IRF-4+CD79a^low^CD20− cells largely resemble the mononuclear tumour cells in classical Hodgkin's lymphoma, both immunophenotypically[32] and morphologically. They may well represent their normal counterpart. While classical Hodgkin's lymphoma has been related to the germinal centre,[33] these CD30+ reactive blasts correspond to postfollicular differentiation. In classical Hodgkin's lymphoma, only

exceptional cases represent an intrafollicular pattern,[34] while the tumour cells are usually located outside the follicle in the mantle zone or T zones.[35] A postfollicular differentiation state may be caused by the nuclear factor κB that is deregulated in Reed–Sternberg cells of classical Hodgkin's lymphoma.[36,37] This molecule is related to the activated B-cell type of diffuse large B-cell lymphoma but not to the germinal centre type.[38]

Reactive extrafollicular B-cell activation enters the differential diagnosis of T-cell expansions in nodular cortical hyperplasia or T-cell lymphomas. In Giemsa-stained sections, the strong basophilia and pale Golgi areas may suggest their B-cell derivation that is confirmed by immunohistochemistry. Diffuse large B-cell lymphoma may also present with a prominent interfollicular pattern. These lymphomas usually represent a 'frozen' differentiation state of centroblasts, immunoblasts or plasmablasts. In contrast, the cases described here exhibit the whole range of differentiation to mature plasma cells and polytypic light chain expression can be proven by immunohistochemistry.

In conclusion, the thorough phenotypic characterization of extrafollicular activation allows the correlation of such morphological observations in human material to immunological processes previously described in laboratory animals. Prominent extrafollicular activation of the B-cell system in the absence of follicular hyperplasia is the morphological correlate of vigorous rapid B-cell expansion, either in primary responses to new antigens or in recall responses of re-stimulated memory B cells.

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