Gastric extranodal marginal zone B-cell lymphomas of MALT type exclusively express toll-like receptor 4 in contrast to other lymphomas infiltrating the stomach

P. Adam1*, B. Schmausser1, M. Göbeler-Kolve2, H. K. Müller-Hermelink1 & M. Eck1

1Pathologisches Institut; 2Medizinische Poliklinik, Universität Würzburg, Würzburg, Germany

Received 6 February 2007; revised 16 September 2007; accepted 17 September 2007

Background: Development and growth of extranodal marginal zone B-cell lymphomas (eMZBCL) of mucosa-associated lymphoid tissue (MALT) type are thought to be highly dependent on Helicobacter pylori and autoantigens. Receptors mediating these effects are not characterised so far. Toll-like receptors (TLRs) recognise bacterial proteins and autoantigens, which results in inflammatory reactions and influences tumour development and growth.

Materials and methods: TLR4, 5 and 9 expressions were evaluated by immunohistology and confocal microscopy in gastric eMZBCL in comparison to other lymphomas infiltrating the stomach.

Results: TLR4 was exclusively expressed on the cell surface in all eMZBCL (n = 19) and not in chronic lymphocytic leukaemia (CLL, n = 12) or mantle cell lymphoma (MCL, n = 10). TLR5 was strongly expressed in CLL and weak in some eMZBCL (15 of 19), but not in MCL. TLR4, 5 and 9 were negative in all the three lymphoma entities.

Conclusions: Exclusive TLR4 expression may enable eMZBCL to interact with H. pylori and autoantigens. Blockade of TLR4 might be a new approach for therapy of eMZBCL of MALT type.

Key words: gastric lymphoma, MALT lymphoma, TLR4, 5 and 9, toll-like receptors

Introduction

Infection of the gastric mucosa with Helicobacter pylori results in chronic active gastritis with the acquisition of a mucosa-associated lymphoid tissue (MALT), the background from which extranodal marginal zone B-cell lymphoma (eMZBCL) of MALT type arises [1, 2]. H. pylori seems to trigger the expansion of the malignant clone and eradication of the bacterium in most cases results in tumour regression [3]. But a direct recognition of H. pylori by the B-cell receptor of the lymphoma cells was not demonstrable. In addition to bacterial ligands, autoantigens, e.g. heat-shock proteins (HSPs), are discussed to drive lymphoma development and growth [4], but the receptors mediating these effects are not characterised so far.

Toll-like receptors (TLRs) as a part of the innate immune system have been shown to recognise bacterial ligands and some autoantigens, which results in the activation of proinflammatory cytokines and chemokines[5]. Recent data also indicate that TLRs influence apoptosis and cell proliferation [6]. Especially, TLR4 has been shown to recognise in addition to bacterial lipopolysaccharides (LPS) some endogenous ligands like HSP [7]. In addition, recent studies demonstrated that some malignant tumours, e.g. gastric carcinomas [8], as well as some lymphomas express TLRs [9, 10]. Therefore, TLRs expressed on tumour cells are possible candidates for interaction of the tumour with bacterial products or autoantigens.

In this study, we investigated for the first time TLR expression in gastric eMZBCL and compared these results with the TLR expression in other non-Hodgkin’s lymphomas [chronic lymphocytic leukaemia (CLL) and mantle cell lymphoma (MCL)] infiltrating the stomach, which grow independently of bacterial or endogenous ligands.

tissue samples and methods

tissue samples

Gastric tissue specimens from 19 patients with gastric eMZBCL of MALT type (12 low-grade and seven diffuse large B-cell lymphomas with eMZBCL component) 12 patients with gastric manifestation of CLL and 10 patients with MCL infiltrating the stomach were included in this study. All tumours were reviewed and classified according to the criteria of the World Health Organisation Classification of Tumours of Haematopoietic and Lymphoid Tissues [11].

immunohistochemistry

Tissue specimens were fixed overnight in 4% buffered formaldehyde solution (pH 7.0) after paraffin embedding tissue blocks were cut into 4-μm sections. For light microscopy, immunohistochemical staining was carried out using antibodies against CD20, CD5, CD23, cyclin D1, AE1/3 and Ki67.
Cases were only included in this study if they fulfilled the morphologic and immunohistochemical diagnostic criteria. For the diagnosis of eMZBCL of MALT type, CD20 positivity of the tumour cells with negativity for CD5 and CD23 was required. Characteristic lymphoepithelial lesions had to be detectable in the staining with the pan-keratin marker AE1/3.

MCL cells showed specific nuclear expression of cyclin D1 and in CLL, a co-expression of CD20, CD5 and CD23 with negativity for cyclin D1 was detectable.

For immunohistochemical detection of TLRs, polyclonal rabbit anti-TLR4 (1 : 500), polyclonal rabbit anti-TLR5 (1 : 1000, both Zymed, San Francisco, CA) and monoclonal mouse anti-TLR9 (1 : 200, Biocarta, Hamburg, Germany) were used as recently described [12]. Briefly, after microwave pretreatment and blocking of non-specific binding sites, primary antibodies were incubated overnight at 4°C. A biotin/streptavidin-peroxidase detection system (Super Sensitive Multilink HRP Detection System, Bio Genex, San Ramon, CA) using 3,3’-diaminobenzidine-tetrahydrochloride solution as substrate was employed as a secondary reagent.

Figure 1. Immunohistochemical staining of toll-like receptor (TLR) 4, TLR5 and TLR9: gastric extranodal marginal zone B-cell lymphoma of mucosa-associated lymphoid tissue (MALT) type showed strong expression of TLR4 (A) and only weak reactivity for TLR5 (B). TLR9 was not expressed (C). In chronic lymphocytic leukaemia (CLL), only TLR5 was detectable (E), whereas stainings for TLR4 (D) and TLR9 (F) yielded no reactivity. In mantle cell lymphoma (MCL), none of the three TLRs was found (G–I; all x1000). Gastric mucosa served as positive control (J–L). Double staining for confocal laser scanning microscopy: reactive CD3-positive (green) T cells within the tumour infiltrate did not express TLR4 (red) (M). In contrast, CD79a-positive B cells (green) of the tumour clone often showed co-expression of TLR4 (red).
Heidelberg, Germany). evaluated with a confocal laser scanning microscope (TCS SP2, Leica, diamidino-2-phenylindole (Sigma, Hannover, Germany). Stainings were gastric eMZBCL (Figure 1N). Reactive CD3-positive T cells, in contrast, were compared it to that of gastric infiltrates of CLL and MCL, which are thought to grow independently of bacterial and endogenous ligands.

In the presented study, all 19 cases of gastric eMZBCL (12 low-grade and 7 diffuse large B-cell lymphomas with eMZBCL component) expressed TLR4 specifically, whereas CLL and MCL infiltrating the stomach did not express TLR4. TLR5 was predominantly expressed by CLL, and to a lesser extent in eMZBCL. Expression of TLR9 was detectable in none of the tested lymphomas. Our data demonstrate a quite different distribution of TLRs between lymphomas infiltrating the gastric mucosa.

The pattern of TLR expression correlates well with the putative model of lymphomagenesis and progression. Tumour growth of gastric eMZBCL of MALT type seems to be highly dependent on bacterial and endogenous stimuli and eradication therapy results in most cases in tumour regression. Whereas the other two lymphoma entities probably grow independently of external stimuli and their clinical course is therapeutically barely not influenceable.

Therefore, it may be speculated that the exclusive expression of TLR4 gives eMZBCL of MALT type the possibility to interact with bacterial antigens and autoantigens. Recently, the relevance of TLR4 in MALT-type lymphomagenesis was underlined by a study determining TLR4 polymorphisms in the peripheral blood [18]. In this study, a negative association of the TLR4 Asp299Gly allele, which is known to diminish the inflammatory response and the susceptibility to develop eMZBCL of MALT type, was found.

In summary, we show for the first time an exclusive expression of TLR4 by eMZBCL of MALT type in the stomach.

Further studies have to demonstrate whether blockade of the TLR4 may be a possible therapeutic approach in preventing development and growth of this specific lymphoma entity.

funding
Deutsche Forschungsgemeinschaft, German Research Foundation (EC 203/1-3).

acknowledgements
The authors thank Eva Bachmann, Sabine Roth and Erwin Schmitt for their excellent technical assistance.

references


