Microenvironment and anti-CD20 based therapies in CLL
Jak, M.

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Summary

The work described in this thesis can be divided into two parts. The first part focuses on the bidirectional interaction of T cells and CLL cells and its consequences on both cell types (chapter 2-3). The second part describes: 1. the mechanisms of direct cell death induced by type I and type II anti-CD20 mAbs in CLL cells and 2. the effect of microenvironment derived signals on the sensitivity of CLL cells to anti-CD20 mediated cell death in vitro and in vivo (chapter 4-5-6).

Chapter 1 introduces the current understanding of CLL cell biology, the effects of mutual interaction of T cells and CLL cells, therapy, different anti-CD20 mAbs and mechanisms of anti-CD20 mediated cell death. Finally, the scope of this thesis is outlined.

In Chapter 2 we compared the effect of two in vitro systems on activation, drug resistance and proliferation of CLL cells. To study the mechanisms of proliferation and drug resistance of CLL cells, robust and reliable experimental systems must be developed that resemble the lymph node microenvironment. An important signal in the lymph node microenvironment is CD40L activation of CLL cells by activated T cells. In this study, the in vitro systems mimic this CLL-T cell interaction: the activated T cell system (CLL cells cocultured with autologous activated T cells) and the 3T40L system (CLL cells cocultured with mouse fibroblasts expressing human CD40L). Activation and drug resistance of CLL cells was induced in both systems. However, proliferation which is an important characteristic of CLL cells in vivo, was only observed in CLL cells cocultured with activated T cells. Importantly, this proliferation was IL-21 dependent. This underscores the important role of T cells in the pathophysiology of the disease. Gene expression of CLL cells co-cultured with either CD40L transfectants or activated autologous T cells was comparable in both in vitro systems. Interestingly they resembled transcriptomes of CLL cells obtained from freshly isolated lymph nodes as to increased NF-κB activity. We conclude that both in vitro systems mimic the in vivo lymph node microenvironment in CLL. However, considering the proliferation induced by activated T cells, this system probably most faithfully reflects the in vivo CLL LN microenvironment.

In Chapter 3 we dissected the underlying mechanisms of increased Treg numbers in CLL. Neither analysis of the T cell receptor (TCR) repertoire nor CD45 isoform expression of Treg from CLL patients provided evidence for chronic (tumor) antigenic stimulation as a possible cause for Treg expansion in CLL. However, both enhanced survival and formation of Tregs was observed in CLL. RT-MLPA expression analysis of 34 apoptosis-regulating genes showed that in comparison to other CD4+ T cells, Treg from both healthy individuals and CLL patients had a high expression of pro-apoptotic Noxa and a low expression of anti-apoptotic Bcl-2. Strikingly, Bcl-2 levels of Treg in CLL patients were significantly higher than in healthy individuals. This resulted in enhanced survival and increased resistance to
drug-induced apoptosis. Furthermore, CLL cells induced Treg in a CD70-dependent manner. The preferential site of Treg formation in CLL in vivo is most likely the lymph node, where CD40-induced upregulation of CD70 on CLL cells takes place. In conclusion, Treg in CLL accumulate by increased survival as result of increased Bcl-2 expression and by enhanced formation facilitated by CD27-CD70 interaction in lymph node proliferation centers.

In Chapter 4 we focused on the mechanism of direct cell death induction in CLL cells by the type I anti-CD20 mAb rituximab. We analyzed the effect of CD40 stimulation, an important microenvironment derived signal inducing drug resistance in vivo, on the sensitivity of CLL cells to rituximab. Whereas CD40 stimulation induced strong resistance to cytotoxic drugs, it sensitized CLL cells to rituximab-induced cell death. This increased sensitivity was specific for anti-CD20 treatment. Rituximab-mediated death in CD40-stimulated CLL cells showed rapid kinetics (within hours) and was caspase- and p53-independent. Rituximab induced Ca⁺²⁺ and ROS-dependent non-apoptotic cell death. By increasing basal ROS production CD40 stimulation sensitized CLL cells to rituximab-induced cell death. Finally, combination treatment of rituximab and fludarabine showed synergistic effects in CD40-stimulated CLL cells. By inducing ROS, rituximab sensitized to fludarabine-induced cell death.

In Chapter 5 we investigated the underlying mechanisms of the type II anti-CD20 mAb (GA101) induced cell death in CLL. Also here, CD40 stimulation sensitized CLL cells to anti-CD20 induced cell death and we explored the basis for this sensitization. GA101 induced cell death occurred without crosslinking. GA101 induced lysosomal cell death in CLL, where lysosomal burst and release of hydrolases into the cytosol lead to non-classical apoptotic cell death. CD40 stimulation increased lysosomal number and activity, thereby sensitizing CLL cells to GA101-induced lysosomal cell death. Finally, the effect of combination treatment with GA101 and different cytostatic drugs on cell death in CD40-stimulated CLL cells was tested. This resulted in strong cell death induction, also in unmutated (UM) and p53-dysfunctional CLL cells. These findings indicate that GA101 has efficacy against chemoresistant CLL.

Chapter 4 and 5 provide a rationale for the treatment of CLL with a combination of cytotoxic drugs and anti-CD20 monoclonal antibodies.

Chapter 6 aimed to define the potential role of CD40 stimulation on type I and type II anti-CD20 induced cell death in vivo. Human CD20TG mice were treated with mouse anti-CD40 mAbs and subsequently either rituximab or GA101 was administered. After 5 days B cell depletion in peripheral blood and secondary lymphoid organs was measured. Also in mice, CD40 stimulation in vitro sensitized to anti-CD20 mediated cell death. CD40 stimulation in vivo resulted in a massive increase in total B cell numbers in peripheral blood, spleen and lymph nodes, which were all depleted after rituximab or GA101 treatment. One exception were mature B cells in bone marrow, where CD40 pretreatment resulted in
resistance to both rituximab- and GA101-induced cell death. Overall, no differences between rituximab and GA101 induced B cell depletion in this system were found. We conclude that for most B cell subsets CD40-CD40L interaction is an unlikely mechanism of resistance to anti-CD20 monoclonal antibodies in vivo. Finally, in vivo treatment with CD40 mAbs should be approached with caution as CD40 triggering in vivo results in a profound increase in B cell numbers, which could be detrimental in patients with B cell malignancies.

Chapter 7 presents an integrated summary and discussion and future approaches towards the treatment of CLL are suggested.