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Unravelling the syndrome of age associated diseases 2:
Targeting autoimmunity with anti-CD20 antibodies to reduce age-associated diseases

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Abstract

The antibody Rituximab that was originally used to treat non-Hodgkin's lymphoma or chronic lymphocytic leukaemia was found to be effective for all autoimmune diseases studied so far. This raises the question whether a successful treatment of autoimmune reactions, even at the subclinical level, can reduce the risk of cardiovascular disease, cancer and other age-associated diseases. Animal experiments and initial clinical observations support this novel therapeutic strategy. However, due to the complex biological processes and the long time required for clinical observations it is challenging to produce clear evidence from randomized double-blinded studies. It will be crucial to prove in the near future, whether surrogate endpoints (e.g., elimination of autoreactive B cells) can accelerate the development of this strategy. The present preclinical evidence is convincing, but the path from a "promising anti-aging molecule" to "accepted drug" will be long and require further research.

1. Therapeutic approaches to ageing and AAD with anti-CD-20 Antibodies

Ageing is associated with an age-related increased number of mutations in all cell lineages capable of proliferation, which appear to play a significant role in age-related inflammation in virtually all organs. The process of cellular senescence is also associated with inflammation (Gewirtz 2014; Hodes, Sierra et al. 2016) most likely also promoting immune reactions including autoimmunity. However, the likely relationship between senescence, mutations, and autoimmunity has yet to be investigated. While numerous strategies to delay or prevent ageing have been explored in experimental and clinical studies (Rattan 2005), none of these have to our knowledge as yet studied or proposed targeting antibodies and autoimmunity in order to ameliorate or slow down the syndrome of ageing and age-associate disorders (AAD). AAD are, in our opinion, not isolated disorders but part of a syndrome of interrelated disorders and interrelated pathogenetic pathways with the acquisition of genetic and epigenetic alterations with age as a major contributing factor. We have proposed the term *"Omega-Syndrome"* for this syndrome of age-associated disorders (Faletti 2019).

Immunosupportive therapies of aging have been proposed (Fulop, Larbi et al. 2007). However, if our hypothesis is correct, that antibodies and autoantibodies play a major role in the pathogenesis of this syndrome, clinical evaluation of anti-CD-20 antibodies, which have shown clinical activity in probably all autoimmune disorders they have been tested in, is warranted (Franks, Getahun et al. 2016).

Long-term clinical observations for more than 15 years of a large number of patients after receiving Rituximab suggest, that these patients rarely develop or suffer from cardiovascular events, show an impressive performance status, and, if they develop secondary or tertiary cancers do remarkably well (Mertelsmann et al. unpublished). In fact, this review article has been prompted by these clinical observations. In addition to doing remarkably well, unexpected benefits of anti-CD20 therapy have been observed in several patients. Two case vignettes may serve as an example.

2. Patient Case Vignettes

- Patient 1: 39 y/o Patient with CLL and plantar warts disappearing after Rituximab therapy

A 40-year old patient with chronic lymphocytic leukemia was diagnosed in 2003. After two years of follow-up without specific therapy the patient received FCR in 2005. He achieved a stable disease with normal peripheral blood counts. However, in 2011 he developed a large number of warts both, his hands and his feet with a significant reduction in quality of life. In 2014, he developed an autoimmune hemolytic anemia. Since this anemia was considered to be part of a reactivation of his underlying chronic lymphocytic leukemia he received four cycles of rituximab. After several months his warts cleared and have not returned while the patient continuous in clinical complete remission.

- Patient 2: 75-year old patient with CLL and disseminated Merkel cell carcinoma

Because of the disease progression of his primary malignancy chronic lymphocytic leukemia the patient received rituximab. In addition, the patient received low-dose chemotherapy as well as radiotherapy to 3 involved areas. The patient entered a clinical complete remission from his Merkel cell cancer. Now, five years later, the patient continues in clinical complete remission. While Merkel cell cancer normally carries a very poor prognosis with the median survival of approximately six months (Nghiem, Bhatia et al. 2016), the unexpected benign course of his course does suggest a beneficial effect of B cell depletion by rituximab.

Merkel-cell-carcinoma is a rare and very aggressive form of skin cancer. An association of this form of virus associated cancers has been observed with chronic lymphocytic leukemia and other B-cell lymphoproliferative disorders (Tadmor et al. 2018).

In both patients, an underlying immune defect was the likely course of a virus-associated disorder. Quite surprisingly, B cell depletion did not worsen the clinical course. In contrast, this treatment apparently restored the immune defect of T cells (most likely) by eliminating or reducing malignant B cells and possibly other types of B cells interfering with T cell Immunity such as regulatory B cells or B cells producing pathogenetic antibodies.

3. Anti-CD 20 Monoclonal Antibodies

Rituximab is a chimeric monoclonal antibody (IgG1) which was developed in the 1990s for the treatment of non-Hodgkin's lymphoma of B-cell origin (Pescovitz 2006). It was first licensed 1997 for the treatment of follicular lymphoma, later for diffuse large non-Hodgkin's lymphoma and chronic lymphatic leukemia. Subsequently, it was shown to be active against all CD 20 positive malignant lymphomas. In 2006 Rituximab was licensed for the treatment of the autoimmune disease Rheumatoid arthritis (RA).

In 2013 it was licensed for the treatment of ANCA associated vasculitis. It is also widely used off-label for the treatment of other autoimmune diseases, even those that were hitherto considered to be T cell disorders such as multiple sclerosis.

Recently, generic and novel fully humanized anti-CD20 antibodies have become available, e.g. Obinutuzumab and Ofatumumab. While there is no cross-resistance in some patients, in general, activity and side-effects appear to be very similar. Recently, Li et al. (Li, Zhang et al. 2015) described a novel multi-component anti-CD20 mAb nanocluster, which demonstrated activity in a Rituximab-resistant B-cell lymphoma, demonstrating that future development of current anti-CD20 strategies might further enhance the efficacy of anti-CD20 strategies.

4. Current Clinical Indications for Rituximab/ Ofatumumab/ Ocrelizumab

Since Rituximab`s approval for the treatment of B-cell lymphomas in 1997, it has significantly improved the clinical course and prognosis of these patients (Pavanello, Zucca et al. 2017; Salles, Barrett et al. 2017). Subsequently, it has been approved for certain autoimmune disorders and furthermore been employed off-label in a very broad spectrum of autoimmune disorders (Edwards and Cambridge 2006).

In certain hematological autoimmune disorders such as immune thrombocytopenia (ITP) and autoimmune hemolytic anemia, Rituximab is highly effective, probably associated with fewer side-effects than with other treatments (Barcellini and Zanella 2011). Unfortunately, there is no approval by the regulatory agencies for many rare indications, since no clinical trials in these rare disorders have been performed and are apparently of no interest to the respective pharmaceutical companies. Since insurance companies in many instances will only pay for Rituximab in approved indications, many patients currently receive more toxic regimens for their disorder, a highly regrettable state of affairs. It is striking that B cell depletion is also of

therapeutic benefit in autoimmune disorders in which a T cell-mediated pathogenesis has been implicated until now. While T cells might still be at the root of pathogenesis, apparently B cell mediated effects dominate the clinical effects, which are responsive to Rituximab.

5. B-cell depletion and Hypogammaglobulinemia induced by anti-CD-20 antibodies

Rituximab targets all CD20 expressing B cells including precursor B-cells, mature, and memory B-cells. However, it does not affect terminally differentiated, long-lived plasma cells and CD20-negative early B-cell precursors (Leandro 2013; Alaibac 2018). Concomitantly, serum immunoglobulins are significantly repressed in most patients following Rituximab therapy, while preexisting plasma cell produced antibodies are not affected (Alaibac 2018). Even ultra-low doses of Rituximab can be therapeutically efficacious (Alaibac 2018). Detailed studies investigating the dose response relationship between Rituximab dose and effects on B cells and other immune parameters are missing. The effect of Rituximab-therapy on circulating B-cell cells and immunoglobulin levels varies from patient to patient even when treated with the same dose. However, it tends to be consistent in the same individual (Leandro 2013). Individual factors including genetic polymorphisms play a role in determining the final extent of depletion (Leandro 2013).

Gudbransdottir et al. (Gudbrandsdottir, Ghanima et al. 2017) report a significant decrease of CD20+- memory B cells and a significant increase in CD5+ B cells, compatible with the induction of regulatory B-cells (Bregs) in patients with primary ITP. They also observe an inverse correlation between platelet number and the proportion of CD27+ memory B cells, suggesting that these cells might be the main culprit in the pathogenesis of this autoimmune disease. In a study of patients with various kidney diseases, Sentis et al. (Sentis, Diekmann et al. 2017) observed in addition to B cell depletion also a significant decrease of CD4+- T-cells as well as of memory T cells with an increase in naïve T cells. B cells tend to recover after approximately 9 months in most patients, however we did not find published data on T-cell recovery after Rituximab therapy.

Taking these findings together, the depletion of CD20+ and CD27+ memory B-cells as well as the reduction of CD4+ T-cells and the increase of naïve T-cells appear to be the major components of this therapeutic pathway. However, immunophenotypic results vary somewhat from publication to publication.

6. Low Risk of Infection in spite of Hypogammaglobulinemia and B-lymphocytopenia

In spite of the significantly reduced number of B cells and immunoglobulin levels, the frequency of serious infections is remarkably low as demonstrated by a retrospective analysis of 3595 patients with rheumatoid arthritis after rituximab treatment (van Vollenhoven, Fleischmann et al. 2015). A reactivation of viral Hepatitis B however remains a concern. Lee et al. (Lee, Park et al. 2018) demonstrated that there is an impact of rituximab dose on hepatitis B virus (HBV) reactivation.

In view of the relatively low incidence of infections in spite of hypogammaglobulinemia and low circulating numbers of B cells, the question arises, whether redundant, possibly damaging immunoglobulins and B-cells are predominantly removed by anti-CD20 therapies, e.g. autoreactive and inflammatory antibodies and B cells.

In some patients with very low levels of B cells and immunoglobulins, the residual B cells and immunoglobulins apparently contain most relevant anti-infective clones, since many of these patients do not exhibit a higher risk of infections during extensive follow-up, even though many of these would be considered severely immunodeficient using quantitative immunological criteria. Apparently, Rituximab removes predominantly autoreactive clones and immunoglobulins, possibly including cells that have acquired functional or genetic hits during ontogeny and the ageing process.

7. A role for B cell depletion and suppression of immunoglobulins with Anti CD 20 Monoclonal Antibodies for the treatment and prevention of AAD: Conclusions and outlook

As we have discussed in this review, mutations, inflammation, and most likely autoimmune phenomena, play a significant role in ageing and AAD. B Cells, antibodies, and autoimmunity appear to be an overlooked player in AAD including cardiovascular diseases and cancer (Figure 1).

Aging and Age-Associated Diseases (AAD)
The Omega Syndrome

Omega Syndrome

- Aging
- CVS
- Cancer
- Neurodegenerative Disease
- Autoimmune diesease
- Etc.
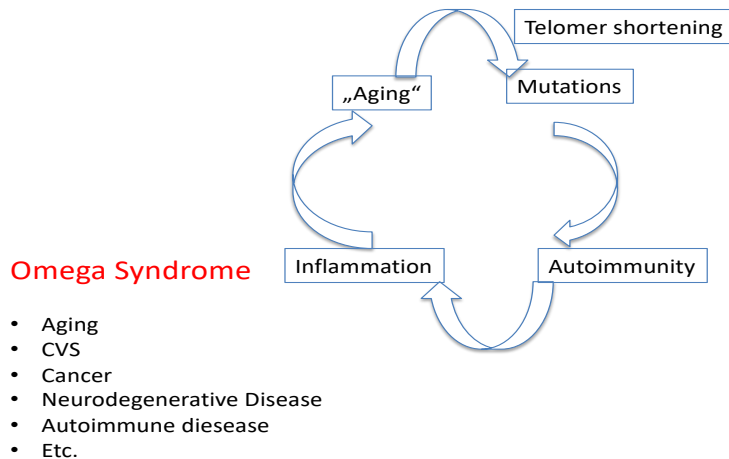
Incidence of MGUS and MBL increase with age supporting the notion that accumulation of genetic changes with age increase the risk of a clone developing with increased survival and proliferation potential as demonstrated for HSC. While there is probably a much higher burden of genetic changes, only those cells that have a proliferative advantage and a survival advantage will be detected as is the case in monoclonal gammopathy or malignant lymphomas. B-cell lymphomas frequently carry an idiotype which recognizes low affinity autoantigens (Sachen, Strohman et al. 2012). Thus, they can be considered as the malignant progression of a preexisting autoreactive B cell clone. Similarly, 33 % of idiotypes in Myeloma have been shown to recognize lysolipids (Nair, Branagan et al. 2016).

Unexpected clinical benefits of Rituximab beyond the treatment of malignant lymphoma and autoimmune disorders observed in patients after Rituximab therapy during long-term clinical follow-up include the following observations and related questions:

- Case Vignettes: Virus-associated Merkel Cell Cancer goes into lasting remission, viral Plantar Warts disappear,
- Clinical Performance Status increases.
- Reduction in the incidence of other AAD, e.g. CVS?
- Survival advantage in clinical trials +/- Rituximab in NHL, only due to NHL effect?

We propose that autoimmunity should be evaluated as a therapeutic target for the prevention and treatment of AAD and that anti-CD20 antibodies could be excellent drugs to evaluate, since Rituximab removes unnecessary, potentially harmful B cells, as well as B cells with genetic alterations like in malignant lymphoma.

As discussed above, in some patients no significant decrease of immunoglobulins and B-cells after Rituximab is observed, which appears to be due to patient-specific factors, possibly genetic polymorphisms. The information regarding a possible correlation of clinical response with the reduction of B- cells and of immunoglobulin levels is very limited to date.

Laboratory studies in Rituximab treated patients with and without receiving chemotherapy should help to clarify the role of

- age-associated B-cells,
- autoreactive B cells,
- mutated B cells, clonal B cells,
- regulatory B cells,
- effects on T Cells
- antibody subtypes, e.g. auto-antibodies,
- Ig levels and risk of MI
- animal Studies with antiCD20 equivalent
- other indicators of inflammation and organ function CRP, pro BNP, Creatinine,
- effects on mutated clones in the hematopoietic and lymphoid compartments.

Long-term clinical observations and regular follow-up will be required to further understand the association of AAD and autoimmunity. Prospective clinical trials lasting for decades appear highly unlikely, because of the logistic challenges involved in such a study and since each patient has and will develop unique constellations of diseases and cofactors during such a long-term follow-up. Case series or cohorts of consecutively treated patients and controls might help to unravel the complexity of the Omega Syndrome of ageing and AAD. The

overwhelming evidence of autoimmunity playing a major role in AAD strongly supports this strategy.

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