Institutionen för medicin, Huddinge

Rituximab-induced neutropenia: clinical and pathophysiological studies

Akademisk Avhandling

Som för avläggande av medicine doktorsexamen vid Karolinska Institutet offentligen försvaras på engleska språket i föreläsningssalen M64, kursrum 5, plan 6 Karolinska universitetssjukhuset Huddinge, 14186 Stockholm

Fredagen den 9 november 2012, kl. 9.00.

av

Daniel Tesfa

Huvudhandledare:
Docent Hans Hägglund
Karolinska Institutet
Institution för medicin, Huddinge

Bihandledare:
Professor Jan Palmblad
Karolinska Institutet
Institution för medicin, Huddinge

Professor Bengt Fadeel
Karolinska Institutet
Institutet för miljömedicin

Fakultetsopponent:
Professor David Dale
University of Washington
Department of Medicine

Betygsnämnd:
Docent Hans Hagberg
Uppsala universitet
Institution för onkologi

Docent Ann-Charlotte Wikström
Karolinska Institutet
Institutionen för biovetenskaper och näringslära

Docent Charlotte Asker-Hagelberg
Karolinska Institutet
Institution för medicin, Solna

Stockholm 2012

Abstract

Rituximab is a monoclonal antibody directed against the CD20 antigen on normal and neoplastic B-lymphocytes. It was originally developed for treatment of lymphomas as a targeted therapy against CD20 positive non-Hodgkin lymphomas (NHL). More recently, its use has expanded into patients with rheumatic diseases. Consistent with this trend, late adverse events of rituximab are appearing, one of these is rituximab induced neutropenia also called late-onset neutropenia (LON). It is defined as an unexplained absolute blood neutrophil count (ANC) < 1.5 x 10^9/L occurring 4 weeks after termination of rituximab therapy up to one year of the follow-up time. However, incidence, mechanism, predisposing factors and clinical consequences of LON are poorly defined. The aim of this study was to address these questions in rituximab treated patients for NHL and rheumatic disease.

We studied the incidence of LON retrospectively in rituximab treated NHL patients. We found an incidence of 8% and a higher incidence was observed in autologous stem cell transplanted patients (Paper I). In this study we observed maturation arrest at the (pro)myelocyte stage of granulopoiesis in the bone marrow (BM) implying a selective depletion of granulocytes. There was no incidence report in rheumatic patients and hence we expanded our studies into this patient group (Paper II). We found similar incidence figure. However, the clinical course of LON was different and it was associated with a higher risk of infections. Moreover, flow cytometry studies on peripheral blood showed that LON patients had pronounced and longer B-lymphocyte depletion compared with non-LON matched controls. Lower IgM levels were evident in LON patients. Thus, the levels of B-lymphocyte depletion and IgM levels may identify patients at risk. Subsequently, we tried to define genetic factors for LON by analyzing polymorphisms affecting B-lymphocyte depletion and production (Paper III). Here, we studied the role of Fc gamma receptor (*FCGR*: *FCGR2A* 131 H/R, *FCGR2B* 232 I/T and *FCGR3A* 176 V/F) and B-lymphocyte activating (*BAFF*: -871C/T) gene promoter polymorphisms for the development of LON. The *FCGR3A* 176V allele was correlated with the occurrence of LON and each V allele was associated with 4-fold increase of odds-ratio for LON. Moreover, patients with this genotype had a longer time to flare of rheumatic disease. Surprisingly, patients who developed LON had also a longer time to flare demonstrating a novel correlation between LON and clinical response. In Paper IV, we tried to elucidate mechanisms of LON. We included rituximab treated NHL patients prospectively. BM and blood samples were obtained at the detection of LON. A pronounced B-lymphocyte depletion in LON patients was also evident during the LON period and this coincided with significant raise in serum BAFF levels compared to non-LON matched controls. Furthermore, BM studies revealed a selective depletion of granulopoiesis (maturation arrest at the (pro)myelocyte) stage during complete B-lymphocyte depletion.

In summary, our studies add to our understanding of LON as a distinct entity. The identification of risk factors such as levels of B-lymphocyte depletion and IgM, and possession of the high affinity *FCGR3A* 176 V allele might be helpful in future clinical practice. Moreover, this genotype as well as the presence of LON were also related to a better clinical outcome. It is, thus, tempting to suggest that LON is a good prognostic factor, but that remains to be proven in a larger prospective studies and lymphoma patients. Finally, our mechanistic studies highlight the interdependence of lymphopoiesis and granulopoiesis which might be orchestrated by BAFF.

 ISBN 978-91-7457-925-3.