SUMMARY AND CONCLUSIONS

The transmission of the human immunodeficiency virus (HIV) by bloodproducts was a disastrous complication of substitution therapy for haemophilia A. Introduction of viral inactivation procedures provided effective methods to prevent HIV seroconversion. Nevertheless, hepatitis C virus (HCV) appeared not to be completely inactivated and administration of factor VIII concentrates showed to be associated with immune abnormalities in HIV negative haemophilia A patients.

The introduction of murine monoclonal antibodies for the production of ultrapure factor VIII concentrates in combination with viral inactivation procedures was the next step to further improve the safety of substitution therapy. The risk of viral transmission has been minimized in this way. Furthermore, immune abnormalities have been reported to be absent in a cohort of previously untreated patients receiving these ultrapure concentrates. This thesis deals with the immune abnormalities in HIV negative haemophilia A patients who had been treated with intermediate purity factor VIII concentrates for approximately nine years, and their course after treatment had been changed to a monoclonal purified product.

An overview from the literature of the observed immune abnormalities in HIV negative patients with haemophilia A is given in chapter 1. An attempt was made to relate the reported immune abnormalities to the steps that can be distinguished in the normal immune reaction. Thus, various steps of the normal immune response that are affected can be identified. Most of the reported abnormalities regarded lymphocyte subsets and their function, both by in vivo or in vitro testing. A multifactorial cause as explanation for the immune abnormalities seems to be most likely. However, despite numerous studies on this subject the knowledge still remains fragmentary.

Aim of the studies in the thesis was to identify immune parameter abnormalities in a group of multitransfused patients with severe haemophilia A, all treated consecutively with an intermediate purity and a heat-treated intermediate purity factor VIII concentrate, and the course of these abnormalities after treatment had been changed to a monoclonal purified product. Analysis of specific changes in lymphocyte subset compilation might clarify the underlying mechanism of the abnormalities found.

First, the efficacy and safety of the monoclonal purified factor VIII concentrate were assessed during five year follow-up (chapter 2). The monoclonal purified factor VIII concentrate showed to be similarly effective as compared with the previously administered concentrates. No HIV infection, no factor VIII inhibitor and no allergic reactions were demonstrated. For the other viruses tested, most patients showed serologic evidence of previous infection at start of the study. Elevated levels of liverenzymes were seen in most patients at any time, these levels fluctuated in time in individual patients. Conclusions on the course of liverenzymes for the whole
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group could not be drawn. The appendix reports on human anti-mouse immunoglobulin G (HAMA) formation in one patient concomitant with treatment with the monoclonal purified concentrate, probably due to the small amount of murine anti-factor VIII present in this product.

The course of lymphocyte subsets during two years after treatment changed from an intermediate purity factor VIII concentrate to the monoclonal purified product is shown in chapter 3. During treatment with the ultrapure concentrate a decrease of HLA-DR expression on non B-lymphocytes during the first year, and a decrease of CD4-CD8 ratio over two years was seen. The decrease in HLA-DR expression is compatible with a decreased immune stimulation. No satisfactory explanation is available for the lowering of the CD4-CD8 ratio. Furthermore, a remarkable increase in the number of platelets during follow-up was observed. This might also reflect a lower level of immune stimulation.

In chapter 4 the relation between CD4-CD8 ratio and the consecutively administered concentrates is analyzed. The homogenous group of previously treated patients showed an increase in CD4-CD8 ratio when they received a heat-treated intermediate purity factor VIII concentrate and a temporary decrease after this product had been replaced by an ultrapure concentrate, these changes were apparently related to the subsequently applied concentrates. This finding provides supportive evidence that immune modulation may depend on the specific concentrates that are used.

Supportive evidence of the supposed relation between concentrate purity and immune abnormalities was obtained by comparing two groups of patients that differed only with respect to purity of the factor VIII products used. The results of this study are summarized in chapter 5. One group had been exclusively treated with cryoprecipitate and the other group had been treated with the concentrates as described above. Both patient groups showed immune abnormalities as compared with healthy matched controls. Elevated numbers of T-lymphocytes and increased numbers of HLA-DR expressing T-lymphocytes were found only in patients who received concentrates. Since all patients were positive for hepatitis C virus differences between groups cannot be explained by infection with this virus.

To define the part of the immune reaction that is involved in the abnormalities found, a more extensive lymphocyte subset analysis was performed as described in chapter 6. Patients, compared with age-matched-controls, showed elevated numbers of T-lymphocytes and an increase in the number of CD8 positive cells expressing activation markers HLA-DR, CD25, CD38 and CD71. This might be the result of chronic viral infections. However, considering the results, reported in chapter 5 it is less likely that hepatitis C virus is the cause of an elevated number of T-lymphocytes and/or increased activation marker expression on T-cells. A remarkable observation was an age related elevation of the number of CD4-CD45RO double positive lymphocytes in patients, which was not demonstrated in controls. This finding might reflect the large amounts of foreign proteins infused during for many years before treatment had been changed to a monoclonal purified product.

During immune activation elevated serum levels of the soluble IL-2 receptor (sIL-2R) may indicate the proliferative phase of the immune response and serum levels of soluble CD8 (sCD8) the effector phase. Serum levels of sIL-2R and sCD8 before, and at two and five years of changing treatment to the monoclonal purified concentrate are described in chapter 7. sIL-2R and sCD8
Levels were elevated in patients treated with an intermediate purity concentrate. A decrease in sIL-2R became evident only after five years treatment with the ultrapure product, while sCD8 levels remained unchanged. These findings suggest a change in the proliferative phase of the immune response without a similar change in the effector phase.

Conclusions

1. The monoclonal purified factor VIII concentrate is efficacious in previously treated haemophilia A patients. It is probably as effective as the less pure products used before in these patients, as far as this conclusion is permitted from a historical comparison.

2. During treatment with this monoclonal purified factor VIII concentrate no HIV transmission, allergic reactions and factor VIII inhibitor formation were demonstrated in the multitransfused haemophilia A patients. Human anti-mouse immunoglobulin G concomitant with the use of the monoclonal purified factor VIII concentrate was seen in one of 22 patients without any clinical features. Overall, preexistent abnormalities of liverenzymes remained unchanged over a period of five years.

3a. Abnormalities due to treatment with intermediate purity factor VIII concentrates in the well described homogenous group of HIV negative haemophiliacs comprised of changes in CD4 and CD8 positive lymphocyte subsets, expression of stimulation marker HLA-DR on T-lymphocytes, and elevated serum levels of soluble IL-2 receptor and soluble CD8.

3b. During the first year of treatment with the ultrapure factor VIII concentrate a decrease of HLA-DR expression suggests a lower level of immune stimulation. A decrease in sIL-2R serum levels after five years, while sCD8 levels remained unchanged, suggest activation in the effector phase that coincides with normalization in the proliferation phase. Besides, the course of CD4:CD8 ratio in this patient group seemed to be related to the purity of the two consecutively applied concentrates.

3c. Immune abnormalities found in patients with haemophilia A who had been treated exclusively with cryoprecipitate were comparable with the findings in patients treated with intermediate purity concentrates, except for differences in numbers of T-lymphocytes and expression of stimulation markers on T-lymphocytes. These differences are not likely to be caused by hepatitis C virus.

3d. Lymphocyte subsets analysis at five years of treatment with the monoclonal purified product showed abnormalities that consisted of elevated numbers of T-lymphocytes, and CD8 positive subsets that express activation markers. Both result from stimulation of the immune system. An age related elevation of CD4 memory cells in the multitransfused HIV negative patients might reflect the amount of foreign proteins infused over many years.

Despite available evidence of immune abnormalities in HIV negative haemophiliacs, and more specifically evidence of stimulation of the immunecsystem, by now there is no convincing evi-
dence that these findings are of clinical significance. This lack of clinical consequences might in part be covered because of the high rate of HIV related morbidity and mortality in the haemophilia population. But, as long as such evidence of the clinical consequences of immune abnormalities is not available, the choice of a factor VIII concentrate for substitution therapy in HIV negative haemophiliacs should primarily be based on the viral safety of the product. The processing of ultrapure products has showed to be contributive only with this respect.