Haematology and Blood Transfusion Vol. 31
Modern Trends in Human Leukemia VII
Edited by Neth, Gallo, Greaves, and Kabisch

Bone-Marrow Purging with Monoclonal Antibodies and Human Complement in ALL and AML

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High-dose treatment followed by infusion of histocompatible allogeneic bone marrow has been shown to be curative in many patients with acute leukemia. Inherent in this treatment modality are a number of problems, however. Most prominent among them: graft versus host disease or, when using T cell-depleted marrow, graft failure. In addition, age restriction, conventionally applied to minimize morbidity and mortality, reduces the application of allogeneic bone marrowtransplantation to, on the average, about one third of those with the disease, and of these only about one in three will have a suitable donor.

Autologous bone marrow support may circumvent some of these problems and restrictions and, under certain conditions, be more effective than currently available conventional treatment protocols. In autologous bone marrow transplantation one of the major problems is the danger of reinfusing residual clonogenic leukemia cells. Remission is usually conceived to be a situation where bone marrow function is apparently normal but there is residual disease undetectable by conventional techniques. It is therefore probable that at least a few leukemic cells will be included in the bone marrow autograft from the remission patient. The numbers reinfused will nevertheless be relatively low, and with the currently used therapeutic modalities it would seem that the observed relapses after ABMT are not infrequently due to endogenous recurrence rather than to proliferation of reinfused tumor cells. It is therefore difficult to clearly prove the additional beneficial effect of bone marrow purging in autologous transplantation.

For this reason the safety requirements for any purging protocol must be particularly stringent. The cytotoxic effects have to be reproducibly selective and the procedural handling as simple as possible. The easiest way to achieve this is incubation with specific monoclonal antibodies which have the capacity to lyse their respective target cells, together with autologous serum as a complement source.

Lysis with MoAbs and human serum makes the purging extremely easy and reproducible and eliminates any foreseeable risks otherwise potentially introduced by the use of heterologous sera as a complement source. It can also be better controlled than more drastic and less specific procedures such as purging with pharmacologic agents. We therefore have concentrated our efforts on finding monoclonal antibodies or mixtures thereof which have that capacity.

Among acute leukemias, purging with monoclonal antibodies has so far been restricted to ALL. For AML no immunology-based techniques were available because of the lack of a suitable specific monoclonal antibody.

In our laboratory as well, the first protocol developed for the elimination of leukemic cells with monoclonal antibodies and human complement was a purging protocol for ALL cells [1]. In this protocol lysis of ALL blasts is induced with a cocktail of

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Table 1. VIM2-reactivity of AML-CFU in AML patients

Patient no.	FAB classification	VIM2 reactivity[a]
1	M2	−
2	M4	+
3	M4	+
4	M4	+
5	M4	++
6	M1	++
7	M1	++
8	M1	++
9	M1	++
10	M2	++
11	M5	++
12	M2	++
13	M1	+
14	M4	+
15	M1	−
16	M2	−
17	M2	−
Normal CFU-GM		−

[a] Bone marrow cells from AML patients and healthy controls were stained with VIM2 antibody in indirect immunofluorescence and then sorted with a fluorescence-activated cell sorter into three fractions of relative fluorescence intensity, i. e., negative (−) weakly positive (+), and intensely positive (++) cells. These separated fractions were inoculated in colony culture to determine the distribution of AML-CFU as a function of antigen density expression in each of the cases.

three IgM-type monoclonal antibodies (termed VIB-pool). These are directed against the CALLA (CD10) antigen (VILA1 antibody) and against two different epitopes of the CD24 surface structure (VIBC5 and VIBE3 antibodies). The purging efficiency was evaluated with leukemic cell lines of the common ALL types (Reh6 and Nalm6) and with blast cells from common ALL patients. Optimal lysis was obtained with antibody and human serum concentrations as low as 1 μg/ml and 7% respectively. As a standard purging protocol we proposed one 20-min incubation at room temperature with antibody, followed by two 30-min incubations at 37 °C with 25% human complement. In dye-exclusion tests 99% purging efficiency and in clonogenic assays detecting elimination of up to 5 logs of clonogenic tumor cells 99.99% (=4 logs) purging efficiency was achieved. Treatment with VIB-pool and human complement had no negative effect on the growth of normal hemopoietic progenitor cells CFU-GM, CFU-E, and BFU-E.

Based on these encouraging results we next screened all our monoclonal anti-leukocyte antibodies for lytic efficiency with human complement [2]. It turned out that some anti-myeloid antibodies also had the capacity to lyse their respective target cells in the presence of human complement.

The most interesting of these antibodies is certainly the broadly reactive anti-myeloid antibody VIM2 [3]. This antibody reacts in >90% of acute myeloid leukemias with a considerable proportion of blast cells. It is not expressed on day-14 CFU-GM cells [4], but the clonogenic leukemia cells (AML-CFU) seem to be VIM2 positive in a considerable proportion of AML patients [5] (Table 1).

Also cytolytic with human complement are the anti-myeloid antibodies VIMD5 [6] and VIM8 [7]. Like VIM2, they are not reactive with day-14 CFU-GM or with CFU-E or BFU-E cells [4] and can, although not as frequently, be found on AML blast cells [6, 7]. We therefore prepared a cocktail of these

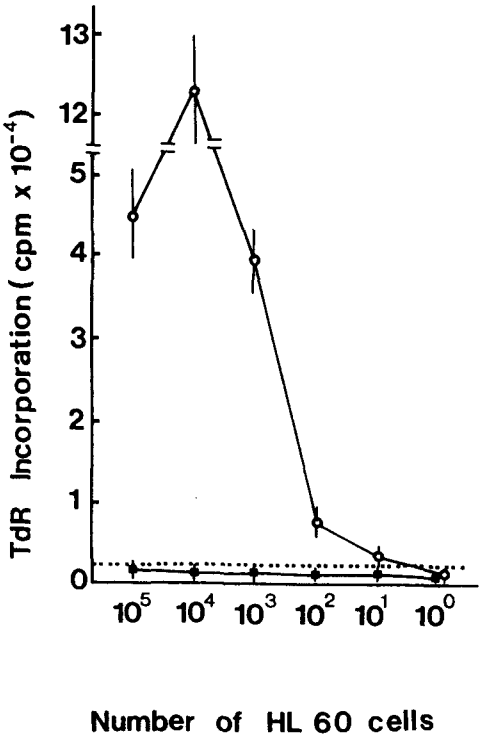

Fig. 1. Proliferation of malignant myeloid cells (HL-60 cells) after treatment with a cocktail of three anti-myeloid monoclonal antibodies (VIM2, VIMD5, VIM8 ■——■) and control antibody (VILA1 o——o) respectively, in the presence of human complement. Cell suspensions were first incubated for 20 min at room temperature with monoclonal antibody. After that, two rounds of complement treatment for 30 min each were performed. The cells were then washed, resuspended in the original volume, and seeded in 96 well microplates at different cell concentrations ($1–10^5$ leukemic cells/well). Plates were cultured for 4 days and proliferation was measured by ^{3}H-thymidine uptake

three antibodies (VIM2, VIMD5, and VIM8) and tested the lytic efficiency of this cocktail in a model system using HL-60 cells as target cells (Fig. 1). As can be seen, the purging efficiency in the model system is quite impressive. More relevant experiments to evaluate the effect of this treatment on clonogenic tumor cells (AML-CFU) in individual AML patients must still be done, however.

Acknowledgement. This study was supported by the *Mediz. wissensch. Fonds des Bürgermeisters der Bundeshauptstadt Wien.*

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