Concise Review

Minimal Residual Disease Post-Bone Marrow Transplantation for Hemato-Oncological Diseases

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Abstract. The detection of minimal residual disease (MRD), which is important in cancer treatment, gained special significance in bone marrow transplantation (BMT) due to the possibility not just to detect but recently also to prevent, treat and reinduce remission in patients that relapsed post-BMT by immunotherapy. The various modern techniques of MRD detection are described including cytogenetics, analysis of restriction fragment length polymorphism, variable number of tandem repeats by Southern Blot or polymerase chain reaction (PCR), microsatellite sequences, PCR amplification products of the Y chromosome or the Amelogenin gene, quantitative PCR and fluorescence in situ hybridization. The role of MRD detection in refinement of indications for BMT, autografting, prediction of relapse, adoptive immunotherapy, mixed chimerism in nonmalignant diseases and in solid organ transplantation is discussed. *Stem Cells 1996;14:300-311*

Introduction

The importance of the detection of minimal residual disease (MRD) in cancer is high since many patients achieving a complete remission (CR) might harbor malignant cells below the limits of detection by standard diagnostic techniques. This has obvious significance for autologous stem cell transplantation. On the other hand, some patients may undergo bone marrow transplantation (BMT) or other forms of aggressive treatment despite the fact that no tumor cells remain in the body.

The detection of small amounts of residual malignant cells has gained vast importance recently, especially in the BMT setting, where management decisions and eventual disease outcome may be influenced by quantifiable levels of MRD in the bone marrow (BM) before or after BMT.

The concepts of chimerism and tumor relapse are used interchangeably in some situations. Sophisticated techniques used for the detection of MRD are very useful for the evaluation of stable engraftment with complete donor chimerism, mixed chimerism where donor and host cells coexist at a certain ratio, graft rejection (takeover of normal host cells) or relapse (the reappearance of malignant cells). Early intervention with cell-mediated immunotherapy may salvage the graft, prevent relapse and reinduce remission.

The most challenging question is whether modification of treatment according to the information provided by such methods can improve the cure rate.

We hereby review the modern techniques of detection of MRD and try to describe the various clinical implications of these methods.

Methods for Detection of MRD

Nonmolecular Methods

Detection of malignant cells based on their morphological features using light microscopy has a limited sensitivity of only 1 to 10%.

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Cytogenetic Markers

The sensitivity of chromosomal analysis depends on the number of metaphases examined. Thirty metaphases must be examined to exclude 10% chimerism with 95% confidence, and 59 metaphases to exclude 5% chimerism with 95% confidence [1]. Occasionally, it is difficult to obtain enough metaphases, especially in the post-transplant period. Also, there may be a proliferative advantage for donor or host cells which would provide misleading results. It is estimated that the sensitivity of this technique is 1 to 10%.

Red Blood Cell Antigens

Red blood cell phenotypes are highly informative genetic markers. A minor population of red blood cells can generally be detected by standard hemagglutination methods when their percentage is as low as 1 to 5% of the total. The fluorescent microsphere method may increase the sensitivity of this assay to one positive cell per 10,000 negative cells (0.01%) [2]. The method analyzes, however, only one cell lineage and the pretransplant phenotype may be difficult to determine should the patient have undergone transfusions. Post-transplant transfusions usually make the exclusion of mixed chimerism impossible for four to six months following BMT, because some transfused red blood cells are detectable for as long as 150 days.

Clonogenic Culture Assays

Clonogenic culture assays detect not only whether malignant cells are present but also if the cells have the ability to induce relapse in the patient. A number of studies has demonstrated that clonogenic malignant cells could be grown from morphologically normal BM [3-5]. Patients with non-Hodgkin's lymphoma (NHL) who were infused with autologous marrow containing lymphoma cells that could be grown in culture had an increased incidence of relapse [6]. Culture assays are capable of detecting one malignant cell among up to 10^5 normal cells. The major technical disadvantage of these assays is their low plating efficiency.

Immunophenotyping

Immunologic analysis of cell populations is limited by the paucity of tumor-specific surface antigens. *Moss et al.* [7] used monoclonal antibodies which react strongly with neuroblastoma cells, but not with normal BM cells, for immunoperoxidase staining achieving a high sensitivity of 10^{-5}. The existence of informative leukemia-associated composite phenotypes by three-color flow cytometry was established by *Griesinger et al.* [8] in acute lymphoblastic leukemia (ALL) and by *Baer et al.* in acute myeloid leukemia (AML) [9]. This method is based on abnormal patterns of coexpression unique for each patient. The sensitivity of this technique is 10^{-5}. Another example is the use of anti-idiotype antibodies directed against Ig molecules produced by B-lineage leukemias and lymphomas. The sensitivity of this approach is defined as 0.1%, since most lymphoma idiotypes are also expressed in a small proportion of normal lymphocytes.

Molecular Methods

Restriction Fragment Length Polymorphism (RFLP)

DNA sequence polymorphism is an informative method of distinguishing donor and host cells. Conventional RFLP may be created by either the loss or gain of the restriction enzyme cleavage sites, or by insertion or deletion of DNA between restriction sites [10]. The likelihood that a particular probe will reveal distinct alleles in recipients and donors depends on the number of alleles and their distribution in the population. The sensitivity of this method using Southern transfer hybridizations is such that a minor population of DNA may be detected when it is approximately 1 to 10% of the total [10, 11].

Southern Blotting for Detection of Clonal Genetic Markers

DNA alterations associated with neoplastic transformation, or during normal differentiation of lymphoid cells, serve as clonal markers. They are easily detected by the increasing number of probes specific for genes rearranged in various chromosomal abnormalities, or clonal markers such as Ig or T cell receptor (TCR) gene rearrangement. The use of Southern blotting for the detection of MRD is limited due to its sensitivity (1%), and to the fact that it is laborious and time-consuming. Other types of genetic markers that can be studied using Southern blotting are the variable numbers of tandem repeats (VNTR). This technique applies the variation in the number of tandemly repeated sequences in

different alleles at hypervariable regions in the human genome. These are called minisatellites or VNTR loci. The length variation can be detected using restriction endonuclease which does not cleave the repeat unit, thus giving rise to a set of stable inherited genetic markers in such a loci. DNA polymorphisms, due to the variation in the numbers of short tandem repeats, are defined by synthetic oligonucleotide probes [12]. Using Southern blot analysis or in-gel hybridization, these oligonucleotide probes are capable of detecting mixed chimerism when the minor population of DNA is present at 1 to 2% of the total leukocyte DNA [13].

Polymerase Chain Reaction (PCR) for Detection of Clonal Genetic Markers

The application of PCR for the amplification of specific DNA or RNA sequences present in neoplastic cells but not in normal host cells, has a very high sensitivity with a detection range limit of 10^{-5}-10^{-6}. PCR was first used to detect molecular markers in human malignancies by *Lee* and colleagues [14], who amplified the t(14;18) translocation in neoplastic cells of patients with follicular lymphoma. The BCL2-IgH PCR has proved particularly useful for assessing the efficiency of marrow purging for autograft treatment of NHL. The submicroscopic deletion in chromosome 1 resulting in juxtaposition of the *sil* and *tal* genes in T-ALL thereby creates a tumor-specific, PCR-amplifiable site, with the potential to assess MRD status in a significant portion of T-ALL. Other examples for leukemia-specific DNA sequences amplified by PCR in detecting MRD are DEK/CAN in t(6;9) AML and PBX1/E2A in t(1;19) ALL.

The physiological rearrangement of Ig and TCR during lymphoid differentiation creates clone-specific sequences which can be followed by PCR [15, 16]. The amplification products are sequenced for the construction of oligonucleotides specific for the malignant cells of each patient. However, potential instability of the configurations of the rearranged gene segments and the variability of sequences within tumor populations may limit the usefulness of this approach for assessment of MRD [17].

The reverse transcriptase (RT)-PCR approach was first applied to detect the *bcr-abl* fusion transcript resulting from the juxtaposition of the *bcr* and *abl* genes on the Ph chromosome following t(9;22) translocation in chronic myeloid leukemia (CML) and ALL cells [18]. In addition to contamination of the PCR products which can be minimized by careful laboratory work, two major shortcomings must be considered: 1) The association between certain translocations and specific pathological entities is not always consistent. This is exemplified by the detection of t(14;18) in large cell lymphomas which evolve from follicular lymphomas and rarely in Burkitt's lymphoma [19]. The existence of t(8;14) in Burkitt's lymphoma as well as in large cell lymphoma [20], and the t(2;5) in Hodgkin's disease as well as in anaplastic large cell lymphoma (ALCL) [21] and in reactive diseases [22], also demonstrates the nonspecificity of chromosomal translocations, and 2) other chromosomal translocations were detected in nonpathological states like t(14;18) in follicular hyperplasia [23], or *bcr-abl* rearrangement in normal individuals [24].

PCR of Minisatellite (VNTR) Sequences

The application of PCR simplifies and quickens the analysis of VNTRs and therefore has become the preferable method of analysis of these polymorphic sequences. A minor population of DNA can be detected even when its concentration is as low as 0.1% of the total [25].

PCR of Microsatellite Sequences

About 50,000 microsatellite sequences ((TG)n repeats) are scattered all over the genome. The use of oligonucleotide primers complementary to flanking unique sequences enables the amplification of those highly polymorphic markers. *Lawler* and colleagues [26] used seven microsatellite markers to evaluate mixed chimerism in 32 transplant patients. They concluded that although the occurrence of mixed chimerism was not indicative of a poor prognosis per se, sudden increase in the proportion of recipient cells could herald graft rejection or relapse.

Y Chromosome-specific PCR

In approximately 50% of cases of allogeneic BMT, donors and recipients are of different sex. *Offit* and associates [27] used the Y chromosome to distinguish between donor and host cells in 64 patients with CML after T cell-depleted allogeneic (allo)BMT. Although mixed chimerism per se did not predict imminent graft failure or relapse, greater than 25% of normal host cells in the marrow at any time after transplantation had a higher probability of clinical relapse.

Witt et al. [28] used repetitive Y-alphoid repeats to study mixed chimerism in female-to-male grafting and found their detection a highly sensitive molecular test (down to 0.01% of foreign DNA).

PCR-Amelogenin: Improved Single-Step PCR Assay for Gender Identification

Assays based on amplification of a Y chromosome-specific sequence by PCR for detection of gender post-BMT have two major drawbacks: 1) only male cells can be detected, i.e., both false positive and negative results are hard to rule out, and 2) in order to be sensitive enough, either a second round of PCR with "nested" primers or many cycles are necessary to detect very small numbers of male cells. Both are easy sources of contamination. The gender of human cells can be determined by PCR amplification of part of the amelogenin gene (AMG) located on both the X chromosome and its shorter copy on the Y chromosome. Hence, female cells are detected by a single amplification product of 977 bp, while male cells give rise to two products of 977 bp (AMGX) and 788 bp (AMGY) [29]. This method requires a minimum of 250 ng DNA, and nested primers are necessary to increase the sensitivity of this assay. *Pugatsch et al.* have improved the published method and can now detect both male and female cells simultaneously using DNA from as little as one male cell in 10^6 female cells, in a single-step PCR reaction (30 cycles) by the addition of a third primer [30]. AMGY is very similar to AMGX, but lacks close to 200 bp; therefore, a different junction sequence unique to AMGY is present. The authors argued that a primer spanning this sequence should recognize Y molecules only, and therefore not compete with the amplification of AMGX-molecules, hence increasing an AMGY-specific signal. The additional AMGY-specific primer amplifies a third product of 218 bp (AMGY2), which is male-specific and not detectable in the female controls.

Quantitative PCR

In many instances, it is not just the presence or absence of a particular sequence that is important—the actual amount of that sequence may be important as well. *Cross et al.* [31] developed a competitive PCR titration assay which estimates the number of BCR-ABL transcripts in CML patients in order to monitor MRD after BMT. The assay gave reproducible results and allowed differences in BCR-ABL message levels of half the magnitude to be distinguished. Patients without cytogenetic relapse generally had low or falling numbers of transcripts. Patients who progressed from cytogenetic remission to cytogenetic relapse, and then to hematological relapse had increasing numbers of BCR-ABL transcripts in their blood. The authors conclude that serial monitoring of residual disease post-BMT by estimating the number of BCR-ABL transcripts provides more information than conventional cytogenetics or nonquantitative PCR, and may identify patients in need of therapeutic intervention before onset of overt relapse [31]. *Lin et al.* also reported that quantitative PCR for BCR-ABL is an effective substitute for conventional cytogenetics to monitor CML patients after BMT [32].

Others [33], using a technique of coamplification of target and control polymorphic minisatellites, achieved quantification by relative intensity of the bands to ascertain degree of mixed chimerism after BMT.

A competitive PCR combined with two-step PCR was developed enabling accurate quantitation of residual lymphoma cells carrying the t(14;18) in follicular lymphoma patients post-BMT, demonstrating a gradual decline in the number of lymphoma cells within consecutive blood and BM samples. Further research might reveal the clinical relevance of data obtained by that method [34].

Deggerdai et al. developed a semiquantitative method to assess the efficacy of purging the BM of follicular lymphoma patients harboring the t(14;18) undergoing autologous (A)BMT. The method is based on coamplification of cancer-specific target molecules with competitor molecules of known concentration [35].

Two-Color Fluorescence In Situ Hybridization (FISH): BCR/ABL Fusion Gene Detection

Tkachuk and colleagues [36] first described the use of fluorescent-labeled DNA probes to the *bcr* and *abl* genes flanking the site of t(9;22) on the Ph chromosome in hybridization analysis of chromosomes of CML cells. In normal cells, separate signals were observed for the different-colored *bcr* and *abl* probes hybridizing to the appropriate genes on their respective chromosomes. However, in marrow cells from patients with CML, juxtaposition of the probes

due to t(9;22) resulted in a distinct fusion signal detectable in metaphase chromosomes. In addition, a major advantage of this approach is that it can be used on interphase nuclei, obviating the need for arresting the cells in metaphase. It enables the study of hypoplastic marrow with an insufficient number of cells for cytogenetic analysis. It also does not require the use of radioisotopes, and it enables the determination of the copy number of targeted sequences. This technique, however, offers no advantages in sensitivity because the theoretical probability of random alignment of the *bcr* and *abl* signals has been estimated to be 1-2% [36]. In the future, the use of probes for entire chromosome [37] and refinement in technology (especially the use of computerized imaging) are expected to minimize the background noise of this technique.

We recently compared the FISH technique to cytogenetics and PCR in its ability to detect the malignant clone of the Ph chromosome [38]. A linear correlation was found between FISH detection of the BCR/ABL fusion product and routine chromosomal analysis. Detection of the BCR/ABL signal by FISH was observed in all patients showing a positive PCR signal. A significant reduction in BCR/ABL signal was observed post-transplant. The FISH examination was reliable also in the peripheral blood. Although PCR detects DNA sequences specific for a particular neoplasm at a $1:10^6$ ratio level, the FISH may offer several advantages: 1) It has been shown that there are two alternative BCR/ABL translocations in RNA junctions; one or both can appear in the same patient. Therefore, if only one probe is used for PCR, the bcr/abl translocation is sometimes not seen. In contrast, the FISH technique is based on hybridization that occurs between DNA of BCR/ABL probes and the genomic DNA. Therefore, the rearrangement can be detected in both types of translocations. This may explain the negative PCR results and positive FISH detected in one of our patients. 2) PCR is much more prone to contamination and false positive results than FISH. Post-BMT, we detected a low percentage of cells with the BCR/ABL fusion product. The clinical significance of this finding has yet to be determined. This probably reflects the PCR positivity in the first months post-BMT.

We recently described the use of FISH in cases of Ph-negative, PCR positive CML [39]. The *abl* probe showed signals on both chromosome 9 bands q34, while the *bcr* probe hybridized to one chromosome 22 and to one chromosome 9. In this case, as in three other cases recently described [40, 41], the BCR/ABL rearrangement was on 9q34, instead of on the usual location 22q11.

FISH in Sex-Mismatch Transplantation

Previous studies have used the FISH technique for analyzing the chimeric status following sex-mismatched BMT using Y chromosome-specific probes. The false positive rate of Y-positive cells as determined by FISH is 0-2.5%, while the false negative rate is considerably higher, 0-13.3%. By using double-target hybridization with differentially labeled probes for both Y and X chromosomes, the false-positive and negative rates may be theoretically reduced.

Bourhis and associates [42] used FISH on the peripheral blood and BM of 45 recipients of sex-mismatched T cell-depleted marrow grafts for hematological malignancies, using probes to X and Y. The probability of disease-free survival was 62.5% for patients with 5% or less host cells, and 28.8% for patients with more than 5% host cells. However, the follow-up time of the study was too short to draw a definite conclusion regarding the relationship between mixed chimerism and relapse.

The use of FISH technique with sex chromosome-specific probes to detect MRD after sex-mismatch BMT in circumstances where the malignant cells are not characterized by detectable karyotypic abnormalities was recently described [43]. We compared the detection of MRD in CML patients post-BMT using FISH to a tumor-specific marker (BCR-ABL) and a sex chromosome-specific probe. We demonstrated that although a higher percentage of cells of recipient origin could be detected by sex-mismatch probes three months after sex-mismatch BMT, most of them had a normal karyotype, whereas the BCR/ABL fusion product probe was more specific and reliable for detection of residual Ph^+ tumor cells after sex-mismatch BMT. This finding may imply that one should be very cautious in using sex-mismatch probes alone to detect residual tumor cells which lack tumor-specific markers. A larger group of patients and longer follow-up will be needed to evaluate the role of FISH using sex-mismatch probes for detection of MRD after sex-mismatch BMT, particularly because the level at which a

clonally abnormal subpopulation of cells detected after BMT becomes clinically significant is still unclear [43].

Clinical Implications

Upfront Transplantation Decision Based on MRD Findings

The decisions regarding who should be transplanted upfront and who should be transplanted later on is based in most protocols, especially in lymphoma and leukemia, on clinical parameters of the patient's disease at presentation (e.g., initial white blood cell count, cytogenetic abnormalities, presence of extramedullary disease) or response to initial chemotherapy. Assessment of tumor burden and response to therapy by detecting MRD may be a useful and practical tool to guide future treatment including no therapy or BMT. The technical feasibility and means for analyzing hemato-oncological patients for the presence of MRD have been demonstrated above. It is still, however, questionable whether a positive result in any of the in vitro tests predicts relapse. It is possible that a small tumor burden is tolerable and can be controlled by the immune system without progressing to overt relapse.

Prediction of Relapse Post-BMT

The most widely studied setting for relapse prediction using MRD assays has been in patients with CML undergoing BMT. Many investigators attempted to determine whether a positive PCR result following BMT predicts relapse, and which patients are at risk. The results from most reports are conflicting and are based on relatively small numbers of patients at only a single time point following BMT [44-47]. It is commonly agreed that a positive result within the first four to six months following BMT occurs very often (80% of the patients according to some studies) and does not predict eventual relapse. This is probably due to the fact that the cells detected at this point are nondividing and eventually will die or be eradicated by graft-versus-leukemia (GVL) reactions. Many of the residual host cells are stromal cells persisting in the marrow for a long period of time [48, 49]. Recent larger studies evaluated in a systematic way the correlation between positive PCR and relapse post-BMT for CML by checking serial patients' sera and different time points post-BMT [50-52]. Three patterns have emerged: patients who are persistently negative, intermittently positive and persistently positive by PCR. Patients with persistently positive PCR results were found to have a higher risk of relapse than those who were persistently negative [50- 52], and should be treated aggressively by immunotherapy. The intermittently positive group of patients is extremely interesting because the presence of small numbers of tumor cells may predict a high rate of relapse with further follow-up, or may remain silent possibly due to immune-mediated mechanism.

Other researchers who were less conclusive [53] conducted a study of 61 patients who underwent BMT for CML which suggested that late positivity identifies a group of patients at increased risk of relapse but is of little predictive value for individual patients. Although all relapses were preceded by PCR positivity, relapse may occur even 12 months after a PCR negative result. The proportion of PCR negative patients at four months after BMT was found to increase significantly with the severity of acute graft-versus-host disease (GVHD), but not subsequently, suggesting that acute GVHD suppresses rather than eliminates the leukemic clone and that these cells may become active again at a later date [53].

MRD is more common in patients who have mixed T cell chimerism after BMT for CML [54]. This suggests that mixed T cell chimerism may be a marker for abrogation of GVL, activity which is thought to be pivotal in eradicating MRD after BMT for CML.

Total cure is only achieved when molecular remission defined as PCR undetectable disease is present. However, the significance of PCR-detectable disease at some time during the follow-up is not yet clear. More accurate serial quantitation would clarify the precise MRD status in leukemia patients and might allow for more accurate prediction of relapse than conventional cytogenetics or nonquantitative PCR [31, 32, 55].

The clinical relevance of detecting the chromosomal translocation t(8;21) in AML by RT-PCR for the AML1/ETO transcript after chemotherapeutic ABMT and alloBMT in long-term remission patients was evaluated [56]. The results indicate the presence of cells carrying the transcript in all patients except the one after alloBMT. While this finding raises interesting questions about the biology of acute leukemia, it limits the value of AML1/ETO RT-PCR for the prediction of impending relapse [11].

As opposed to CML and AML-M2 in which the prognostic significance of PCR evaluation is unclear, PCR negativity should be considered the therapeutic goal in AML-M3 patients because in no cases were residual PML/RAR-α transcripts detected in the remission sera of eight patients in long-term follow-up [57].

Ig gene fingerprinting, by amplification of CDR3 sequences, was used to evaluate MRD in five multiple myeloma patients in unmaintained CR 9-60 months post-alloBMT. All five patients were PCR positive within the first year post-BMT, suggesting that early PCR positivity is common and not predictive of relapse. Three patients studied became PCR negative later on. The ability of the technique to detect clonal evolution was demonstrated by serial studies in another patient who relapsed post-BMT [58].

Adoptive Immunotherapy for CML Patients Relapsing after BMT

Donor-derived effector cells can be administered to durably engraft in allogeneic marrow chimeras. These can confer to the transplanted host the capacity to eradicate residual populations of host-derived CML, and to a lesser extent myeloma, acute leukemias and NHL. This GVL effect is mediated by major histocompatibility complex (MHC)-unrestricted natural killer or lymphokine-activated killer cells, or MHC-restricted T cells [59-62].

The success of BMT in CML patients is limited by the morbidity and mortality caused by GVHD, a complication that can be prevented by the removal of T cells from the donor marrow. A higher incidence of relapse has been reported for T cell depleted grafts compared to unmanipulated BMT, suggesting that the removal of T cells from the donor marrow not only results in a lower incidence of GVHD, but also in a loss of GVL effect [63]. The remission induced with the use of donor leukocyte infusions, sometimes with the addition of biologic response modifiers, for induction of GVL in treatment of relapsed CML patients is associated with about 30% incidence of GVHD. Better results have been achieved when adoptive therapy was given at a state of molecular or cytogenetic relapse and not at overt hematological relapse [60-61, 64-70].

The "relative efficiency" at which molecular relapse can be detected and remission achieved by donor T lymphocytes, and the unacceptable toxicity of unmanipulated BMT, especially in older patients, may suggest an experimental alternative approach. Patients would be offered a T cell-depleted BMT with a low risk of GVHD and transplant-related mortality. Knowing that many of these patients would relapse without intervention [63], regular follow-up with PCR would be performed to detect residual tumor cells. Adoptive immunotherapy with donor leukocytes would then be administered to patients who become PCR positive in order to achieve molecular remission.

Because the success rate of the treatment is dependent on repopulation of the patient's BM by donor hematopoietic cells after elimination of recipient-derived cells, residual donor BM activity at the time of relapse will be an obligatory factor for success of donor-derived adoptive immunotherapy. If no donor-derived BM activity is present at the time of relapse, treatment failure is expected due to two mechanisms: 1) rejection of the transfused donor T cells and 2) elimination of recipient hematopoietic cells which may result in severe irreversible BM aplasia. Detection of residual donor cells at the time of relapse is done by the molecular techniques for detection of chimerism.

Mixed Allogeneic Chimerism as an Approach to Transplantation Tolerance

Although lethal irradiation followed by reconstitution with allogeneic BM leads to fully allogeneic chimerism and successful induction of specific transplantation tolerance in some animal models, two major risks associated with such treatment have limited its applicability to clinical transplantation: GVHD, and reduced long-term immunocompetence, probably due to the failure of donor-derived antigen-presenting cells to cooperate with host MHC-restricted lymphocytes [71]. Contrary to this, animal models producing mixed allogeneic chimerism have few advantages over fully allogeneic chimeras: 1) They provide an intact source of autologous marrow and should engraftment fail, there is a minimal risk for potentially fatal aplasia; 2) hematopoietic cells bearing host MHC molecules provide source accessory cells that can intereact effectively with T cells educated in the thymus, and improve immunocompetence; and 3) the autologous marrow appears to prevent GVHD [71].

Although microchimerism has not yet been intentionally induced in any large-scale clinical

trial, it has been reported to develop spontaneously after alloBMT in some clinical situations including severe aplastic anemia (SAA), thalassemia and leukemia, and possibly with the improvement of detection techniques, it will be reported more. In some unirradiated SAA patients [72], it was noticed that chimerism was stable, but allograft rejection ensued in others. Patients with mixed chimerism who maintained their grafts had a significantly reduced incidence of GVHD. SAA patients receiving irradiation as part of the conditioning therapy tend to have full donor engraftment [73-74]. In leukemic patients, incidence of 10-20% mixed chimerism has been reported after unmodified BMT [75].

The goal of less toxic transplantations achieving mixed chimerism may be obtained by future studies of various chemotherapeutic conditioning which will employ various quantitative techniques in order to assess the degree of chimerism. In case of unstable chimerism, or of overt relapse, more stem cells or donor T cells (in cases of T cell depleted transplantations) may be given without prior toxic chemotherapy.

BMT in Thalassemia and SAA and Detection of MRD

Thalassemic patients tend to develop mixed chimerism post-BMT either due to pre-BMT T cell depletion used at some centers, or to the fact that the thalassemic marrow is hypercellular and packed, and the thalassemic stem cell is less sensitive to ablative chemotherapy than most malignancies [76]. The methods used so far to detect chimerism following BMT for nonmalignant disorders include detection of sex chromosomes in sex-mismatched transplants, analysis of hypervariable regions of the human genome by PCR and typing of human blood groups. The use of β-globin gene mutation as an innovative method to confirm engraftment, detect MRD and establish the degree of chimerism in thalassemic patients post-BMT was recently described [76]. The method, based on allele-specific oligonucleotide hybridization, is highly sensitive and capable of detecting an allele even at a level as low as 1%. In cases where the donor is a carrier for the same mutation, the detection of residual host cells is less sensitive, since the level of the mutant allele in the absence of residual host cells is 50% at the start; therefore, the level of detection begins only at 53-55% mutant alleles, corresponding to 5-10% host cells. Of the 14 patients analyzed over a period of 10 years, seven patients were found to be complete donor chimera and seven patients carried some degree of chimerism. Six of the later patients, all blood transfusion-independent, had donor cells in the range of 70% to 95%, with stable mixed chimerism. The seventh patient had <10% donor cells with only minimal transfusion requirements. Since mixed chimerism was associated with transfusion independence, complete eradication of residual host cells for effective treatment of thalassemia and possibly other genetic diseases may prove not to be a sine qua non [76]. Theoretically, if chimerism is not stable or becomes unstable after a stable period, it is possible to treat the patient with donor cells in order to eliminate residual host cells.

Progressive mixed chimerism post-BMT is predictive of graft rejection in patients transplanted for SAA. Serial monitoring of SAA patients following BMT using a PCR-based assay should be performed, particularly during the period of withdrawal of immunosuppression [77].

Organ Transplantation

The concepts of mixed chimerism and detection of MRD have recently reached the field of organ transplantation. Surgeons puzzled biologists by successfully transplanting organs prior to the advancement of the understanding of the immunology of the procedure. Mild immunosuppression would often prevent graft rejection. It became evident that due to bidirectional migration of cells, the transplanted organ is composed of donor and host cells, and the tissues throughout the body become populated by cells of donor origin for many years to come [78], including donor stem cells followed by donor type hematopoiesis [79]. The assessment of chimeric status following sex-mismatch liver transplantation in 12 patients for a median period of 18 months was recently described [80]. Peripheral blood hematolymphoid cells were hybridized with Y or X chromosome fluorescently labeled specific probes, and the donor-typed hematopoietic cells were enumerated. Those patients with no signs of systemic chimerism were more inclined to acute graft rejection. Inasmuch as balanced systemic chimerism after organ transplantation is of major importance for self-tolerance, our findings may enable us to treat patients after liver transplantation without need for immunosuppression. This may also set the stage for experimental complete BMT combined with organ transplantation from the same

donor, thereby achieving mixed chimerism and tolerance. In addition, adoptive cell-mediated immunotherapy should be experimentally attempted for patients with early graft rejection.

Summary

Advances in molecular techniques have made the follow-up of post-BMT patients for detection of MRD, molecular engraftment, early molecular rejection, chimerism and early relapse feasible. In addition to the important prognostic impact of this information, and the obvious applications for autografting, it may set the stage for a more fine-tuned follow-up. It will enable us in the near future to use the powerful tool of immunotherapy more effectively, at the right time and with the correct cell dose. We may be able to use milder and less toxic conditioning regimens achieving mixed chimerism and tolerance, initiating immunotherapy at the detection point of molecular relapse with a high degree of success and fewer side effects. We may also be able to perform organ transplantation without the need for heavy immunosuppression.

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