

## \* CHAPTER 15

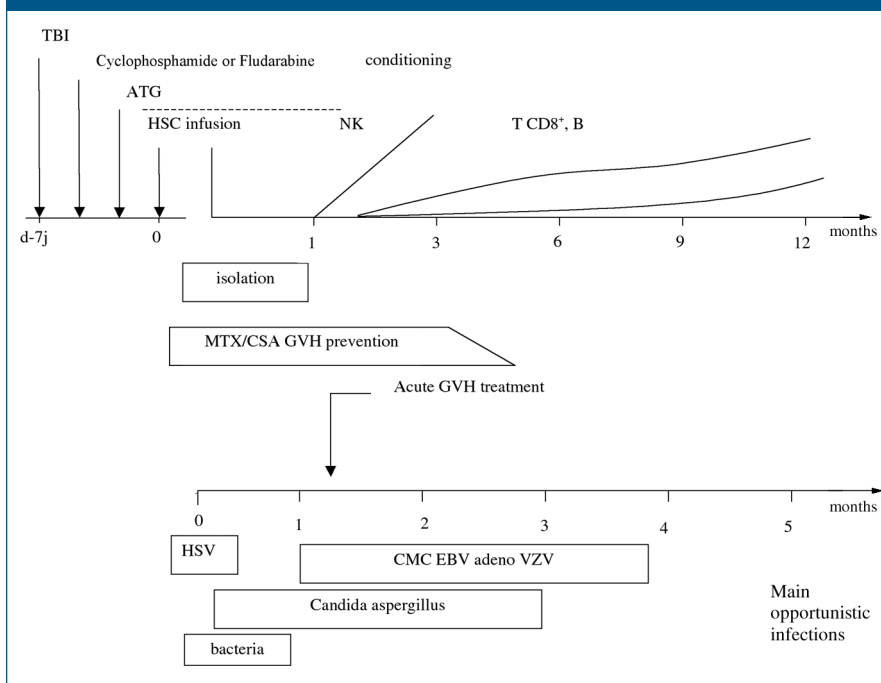
# Immune reconstitution after allogeneic HSCT

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## 1. Introduction

Assessment of the host immune status is becoming a key issue in allo-HSCT, especially in the long-term follow-up of these patients, because severe post-transplant infections, relapse or secondary malignancies may be directly related to persistent immune defects. Immune deficiency leading to an increased susceptibility to infections lasts for more than a year. In relation to the occurrence of infections, the post-transplant period is subdivided in different phases (see Figure 1 and Chapter 10). Although infections that occur in the first month mostly result from a deficiency in both granulocytes and mononuclear cells (MNC), later post-engraftment infections are due to a deficiency in MNC subsets, primarily CD4 T-cells and B-cells. T-cell reconstitution has been extensively studied because of the central role of T-cells in mediating both GvHD, evidenced by the reduced incidence of this complication following TCD, and a GvL effect as shown by DLI. DLI may cure 20–80% of patients with post-transplant relapsed leukaemia and lymphoma depending on the type and

Figure 1: The time course of infections after allogeneic HSCT



extent of the disease. This is one of the most important breakthroughs in HSCT in the last years illustrating the powerful anti-leukaemia effect mediated by allogeneic lymphocytes and the potential of immunotherapy in the treatment of malignant diseases.

## 2. General principles

In transplants performed following myeloablative conditioning regimens, immune reconstitution (IR) will depend upon the ability of the haematopoietic graft to generate *de novo* lymphoid and myeloid lineage cells and on the function of mature cells contained in the graft. Post-transplantation, the different MNC populations reconstitute at different tempos. The first cells to reconstitute (within first 100 days) are those of the innate immune response, granulocytes, monocytes, macrophages and NK cells. In contrast, T and B-lymphocytes remain severely reduced and their function is impaired for several months or years after HSCT. IR of these various lymphocyte populations will be analysed separately with an emphasis on T-cell reconstitution.

## 3. Main factors affecting IR

<b>Host factors</b>	Age, sex, conditioning regimen, initial pathology
<b>Genetic differences</b>	The degree of genetic differences between donor and recipient including HLA, minor histocompatibility Ag and genes associated with immune responses to microorganisms (see Chapter 3).
<b>Source of HSC</b>	The type of HSC, either unmanipulated or TCD BM, PB or CB has an impact on IR. This is an important parameter, for instance recipients of PBSC, who receive at least 10 times more lymphocytes than recipients of BM, have higher lymphocyte-subset counts and fewer infections (1). IR in CB transplants is slow but progressively reaches in the long-term (>2 yrs) even better values than after BM grafts (2).
<b>Post-HSCT events</b>	May in turn have a worsening effect on IR, especially aGvHD and cGvHD, relapse and infectious complications (EBV or CMV viruses, fungal infections, toxoplasmosis) either directly or through drug-related side effects.

## 4. Assessment of B-cell reconstitution

### 4.1. B-lymphocyte phenotyping with B-lineage markers (CD19, CD20, CD21) and activation or differentiation markers (CD5, CD27)

CD19<sup>+</sup> B-cells normalise by one year after transplant. B-cell regeneration may be associated with transient appearance of monoclonal B-cell expansions.

### 4.2. Quantification of serum total IgG, IgM and IgA and of IgG subclasses

After a decline in the first few months after HSCT, levels of specific antibodies to protein Ag frequently encountered after transplantation (e.g. CMV) return to pre-transplantation levels within 1 yr. In contrast, antibodies to protein Ag that are unlikely to be encountered after HSCT (e.g. tetanus, measles, polio) continue to decline. This supports the recommendation of post-HSCT vaccination. Antibody levels in the first year, are affected primarily by pre-HSCT antibody levels in the recipient (3). A persistent defect in IgA, especially in patients with cGvHD explains mucosal infections of the respiratory and digestive tracts. IgG2 and IgG4 subclasses are also deficient in the case of GvHD, accounting for the increased susceptibility to infections, primarily those due to encapsulated bacteria (e.g. *Streptococcus pneumoniae* or *Haemophilus influenzae*). PBSC recipients do not have higher antibody levels than BM recipients.

### 4.3. Vaccinations

Vaccinations with inactivated or conjugated vaccines (see Chapter 10) should be initiated when CD4 and B-lymphocyte counts are sufficient to expect efficacy, usually from 6 months post-transplant onwards.

## 5. NK-cell reconstitution

NK-cells are lymphocytes that act early in the immune response against infection and tumour-transformed cells. Based on phenotyping (CD16 and CD56), they are the first lymphocyte subpopulation to be reconstituted in all graft settings, usually within 3 months.

The genetic organisation and function of NK receptors, either inhibitory or activating, has been unravelled in the past few years. NK-cell receptors are encoded by 2 structurally distinct families of molecules: The killer immunoglobulin-like receptors (KIR) and the lectin-like CD94: NKG2 heterodimers. Every NK-cell expresses at least one inhibitory receptor specific for autologous HLA Class I, thereby ensuring self-tolerance. As KIR and HLA segregate independently and as unrelated individuals almost always have different KIR genotypes, we predict that approximately 25% of

transplants between HLA-identical siblings involve KIR identity and approximately 75% KIR disparity. For transplantation with an HLA-MUD, the frequency of KIR incompatibility approaches 100%. In the haploidentical TCD graft setting, a beneficial effect of KIR disparity on GvL has been evidenced (4). This is an important finding which needs to be extended to other types of grafts and which may change our current criteria of donor/recipient matching based on HLA compatibility (see Chapter 3).

There are still few studies directly assessing NK reconstitution at the level of KIR and lectin-like NK receptors expression level and function (5). This study and others showed that CD94:NKG2A may be expressed earlier than KIR and that most patients reconstitute a donor-type NK repertoire depending on their KIR genotype. Different NK subsets are now more precisely defined and especially the CD56<sup>bright</sup>CD16<sup>-</sup> and CD56<sup>dim</sup>CD16<sup>+</sup>, respectively prone to cytokine production or cytotoxicity. The rapid recovery of NK-cells after graft is due to an expansion of CD56<sup>bright</sup>CD16<sup>-</sup>. The precise role of this NK subset in GvHD and GvL is a key issue in HSCT.

## 6. T-cell reconstitution

### 6.1. Naïve and memory T-cells

Memory T-cells are the first to expand after HSCT; they may be either of donor origin in the case of a non-TCD BM or, in the case of a TCD, originate from host T-cells that have survived the conditioning regimen (6). They respond quickly to previously encountered pathogens, are easier to trigger, faster to respond and enter tissues more readily than naïve T-cells. They are frequently directed towards periodically reactivated herpes viruses, CMV or EBV, which they keep under control. They constitute the majority of oligoclonal T-cell expansions found in healthy adults, especially in the CD8<sup>+</sup> population. They are also less dependent than naïve T-cells upon recognition of self MHC-peptide complexes in their survival and expansion in the periphery.

In the long term, broad immune responses need the reconstitution of a naïve T-cell repertoire able to respond to a broad range of pathogens encountered by the host and to tumour antigens. Reconstitution of this compartment is an ongoing process which requires a functional thymus for the recovery of a complete T-cell ontogeny. The thymus itself may be a target of the alloreactive immune attack with possible consequences on thymic selection, escape of self-reactive T-cell clones and perpetuation of GvHD. This has been well documented in animal models (7) and deserves more insight in humans.

### 6.2. How to evaluate naïve and memory T-cell populations?

The current immunological tests assess naïve and memory lymphocyte populations:

CD45RO for memory T-cells and CD45RA or CD62L for naïve T-cells are the most usual markers. However, naïve T-cells may undergo expansion without phenotypic changes and have a long lifespan, up to 20 yrs. In addition, memory CD45RO<sup>+</sup> T-cells may revert to a naïve CD45RA<sup>+</sup> phenotype, especially in case of persistent infection with herpes viruses. Therefore, other markers should be added to definitely assess naïve T-cells and the different categories of memory T-cells. CCR7, a molecule involved in the homing of T-cells to lymph nodes is especially valuable. A combination of these markers allows the definition of:

- Naïve T-cells: CD45RA<sup>high</sup>CD45RO<sup>-</sup>CCR7<sup>+</sup>CD28<sup>+</sup>
- 2 populations of CD8<sup>+</sup>CD45RA<sup>-</sup> memory cells:
  - CCR7<sup>+</sup> “central memory”, expressing L-selectin (CD62L)
  - CCR7<sup>-</sup> “effector memory”, L-selectin<sup>-</sup>, IL-2 dependent, which migrate to inflammatory sites and secrete IFN- $\gamma$ .

T-cell diversity (“T-cell repertoire”) and thymic function can be directly evaluated. The size of the T-cell repertoire and the extent of T-cell diversity has been measured only recently, the value of about  $25 \times 10^6$  different TCR  $\alpha\beta$  complexes being lower than that was previously estimated. T-cell diversity is contributed by the naïve population and in healthy adults memory T-cells, which account for approximately 1/3 of the total T-cells, contribute to less than 1% of the  $\alpha\beta$ -T-cell diversity. A practical consequence for HSCT is that evaluation of T-cell repertoire diversity reflects the extent of the naïve T-cell compartment. Various approaches known as “Immunoscope” or “spectratyping” may be used. They are based on the size diversity analysis of the CDR3  $\beta$ -chain region as an index of the diversity of the whole  $\alpha\beta$ -T-cell population. T-cell repertoire diversity in allo-HSCT recipients is a function of:

- Number and diversity of infused T-cells with the graft (TCD, age of the donor, source of graft)
- Residual T-cells present in the recipient
- Thymic pathway of regeneration, for which the age of the recipient is the main factor
- Immunosuppressive treatment and complications (GvHD, viral infections).

Overall, early after HSCT (within 6 months after graft) many abnormalities of the T-cell repertoire are demonstrable but are difficult to correlate with the clinical status of the patient. Conversely, later after the graft (after 1 yr at least) and ongoing for at least 2 to 3 yrs post-transplant, it is possible to correlate repertoire disturbance with the occurrence of GvHD, severe infectious complications or relapse. T-cell repertoire reconstitution is delayed in case of TCD or in CD34<sup>+</sup> purified grafts and is improved where there is full donor haematopoiesis. Techniques of TCR  $\beta$ -chain sequencing have clearly separated T-cell clones mediating GvHD and GvL and could be used in the future to monitor GvHD-causing clones in HSCT recipients (8).

The reconstitution and maintenance of a diverse repertoire in the peripheral lymphocyte pool is dependent on the generation of functional thymocytes throughout adult life. These recent thymic emigrants can now be evaluated by measuring the episomal DNA excision circles of the TCR  $\beta$  locus deleted during recombination of the  $\alpha$  locus in all functional  $\alpha\beta$  T-cells (known as "TREC" for T-cell receptor rearrangement excision DNA circles). This *ex vivo* marker of thymic function has been used in allo-HSCT monitoring:

- TREC levels are low until 3–6 months after allo-BMT. Low TREC values are associated with increasing patient age and TCD but mainly with GvHD (9), leukaemia relapse or opportunistic infections
- High TREC levels and a broad T-cell repertoire have been associated with an efficient IR after CB transplantation in the long term (2) although there is some delay in IR in that setting (10).

The thymic function of the recipient before graft could be associated with a more favourable outcome in terms of survival, GvHD and bacterial or viral infections (11). It could be a valuable prognostic factor predicting IR after transplant.

### 6.3. How is the Ag-specific immune response reconstituted after allo-HSCT?

Naïve T-cell reconstitution is a key issue for the long-term recovery of immune responses but memory T-cells are also needed for an efficient and timely response towards pathogens. Therefore, especially in some graft settings (TCD, CBT, HLA mismatch UD) adoptive immunotherapy can be used in an attempt to compensate for the lack of Ag specific immunocompetent T-cells. In order to do this, it is necessary to evaluate patients at risk and to be able to monitor Ag specific immune responses towards pathogens. Herpes viruses (CMV, EBV) are of primary importance in HSCT because reactivation of EBV can result in potentially fatal EBV-associated lymphoproliferative disease and because of the frequency of late CMV reactivation in the host even under pre-emptive therapy. It is possible:

- To monitor EBV and CMV-specific cytotoxic responses by Elispot functional assays or intracellular cytokine staining which are easier to perform than the conventional  $^{51}\text{Cr}$  release cytotoxic assay in a routine laboratory
- To use the tetramer technology to stain directly *ex vivo* CD8<sup>+</sup> T-cells reactive with peptide/HLA complexes and to characterise these cells in terms of phenotype and function. This is a very sensitive method which can stain 1/5000 CD8<sup>+</sup> T-cells (or  $1/5 \times 10^4$  PBMC). It may also be used to isolate Ag-specific T-cells by cell sorting and to expand them *in vitro*. It is becoming a routine laboratory analysis for CMV (12) and EBV (13) specific CD8<sup>+</sup> T-cell responses.

## 7. From monitoring to immune intervention

The combination of these various structural (Immunoscope for TCR diversity analysis, TREC for thymic function) and functional approaches (Elispot, tetramer staining) should enable more precise immune monitoring of patients at risk of relapse or persistent severe infectious complications, particularly in the context of adoptive immunotherapy. The tetramer approach has direct benefits for the identification of patients with impaired CD8<sup>+</sup> specific cytotoxic responses who may be eligible for cellular adoptive immunotherapy against CMV (14) or for CD20-specific MoAb treatment (rituximab) to prevent post-HSCT lymphoproliferative disorder during EBV reactivation (13). The transfer of viral-specific CTL is possible with tetramer-sorted CTL without culture (15).

Based on experimental models, other attempts could be pursued to improve IR and graft outcome:

- Improve thymic function recovery, by growth factors such as KGF, Flt3l or androgen blockade
- Selective depletion of alloreactive T-cells from donor lymphocytes (16)
- Use of minor histocompatibility Ag as tumour Ag to mediate GvL effect, as described for HA-1 minor antigen (17)
- Genetic modification of T-lymphocytes with TK suicide gene (18)
- Haploidentical NK immunotherapy (19)
- Immune modulation through alternative stem cell sources (mesenchymal stem cells), dendritic cells or T<sub>reg</sub> manipulation.

Manipulation of immune system homeostasis to facilitate the emergence of regulatory CD4<sup>+</sup>CD25<sup>high</sup> T-cells (or T<sub>reg</sub>) which have been shown in animal models to control GvHD without impairing GvL (20). In humans, although evidence for the role of T<sub>reg</sub> has been less clear, it appears that an *in situ* defect in these populations could be associated with aGvHD (21). Importantly, T<sub>reg</sub> could be expanded *in vitro* and keep their functional properties, thus being an approach of choice in the future for controlling GvHD.

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## Multiple Choice Questionnaire

To find the correct answer, go to <http://www.esh.org/ebmt-handbook2008answers.htm>

- 1. After allo-HSCT the earliest lymphocyte population(s) to recover:**
- a) NK lymphocytes .....
- b) T CD4+ naïve T-cells .....
- c) T CD8+ memory T-cells .....
- d) All at the same time .....
- 2. Among the following lymphocyte phenotypic marker(s), which one is the most precise to define memory T-cells:**
- a) CD45RA+ .....
- b) CD45RO+ CCR7- .....
- c) CD45RA+ CCR7+ .....
- d) CD16+ CD56+ .....
- 3. Which is the main factor directly affecting thymic recovery after allo-HSCT?**
- a) Sex .....
- b) CMV infection .....
- c) HLA mismatch .....
- d) GvHD .....
- 4. After allogeneic HSCT, the risk of EBV-induced proliferative disease (PTLD) is especially increased in case of which one of the following:**
- a) T-cell depletion .....

- b) Genoidentical sibling donor .....
- c) Sex-mismatch between donor and recipient .....
- d) Aplastic anaemia as primary disease .....

**5. Among the following lymphocyte phenotypic marker(s), which is the most precise to define naïve T-cells:**

- a) CD45RA+ .....
- b) CD45RO+ CCR7- .....
- c) CD45RA+ CCR7+ .....
- d) CD16+ CD56+ .....

## NOTES