

## PHENOTYPIC CHANGES WITH IMMUNOSUPPRESSION IN HUMAN RECIPIENTS

Thomas F Mueller

Department of Medicine, Renal Division, Molecular Immunology – Immunogenetics, Brigham and Women's Hospital, Harvard Medical School, Boston

*'Omnibus dubitandum est' - Descartes*

### TABLE OF CONTENTS

1. Abstract
2. Introduction
3. Physiologic changes in immune phenotypes
  - 3.1. Maturation and differentiation
  - 3.2. Aging
  - 3.3. Lymphocyte compartments
4. Specifics of the transplant setting
5. Immunosuppressive therapy
  - 5.1. Maintenance therapy
  - 5.2. Induction and anti-rejection therapy
    - 5.2.1. Effects on surface molecules by non-depleting agents
    - 5.2.2. Depletion of target cells
      - 5.2.2.1. Mode of action
      - 5.2.2.2. Impact on cell subsets
      - 5.2.2.3. T cell monitoring and dose-adjustment
6. Immune reconstitution and long-term changes
  - 6.1. Regeneration and novel homeostasis
    - 6.1.1. Homeostasis-driven proliferation
    - 6.1.2. Population kinetics and homeostasis
      - 6.1.2.1. Dynamics
      - 6.1.2.2. Plateau, niches, and regulation
      - 6.1.2.3. Functional implications
  - 6.2. Expansion of specific cell subsets
  - 6.3. Accelerated aging of the immune system
7. Summary and perspective
8. Acknowledgement
9. References

### 1. ABSTRACT

The differentiation of lymphocyte subsets plays an important role in transplant medicine. The review describes alterations in the expression of surface molecules on circulating T cells associated with immunosuppressive therapy. Phenotypic changes are reflecting the various stages of T cell development, the degree of thymic involution, and disease- and treatment-associated perturbations. The most pronounced effects on T cell subsets are mediated by depleting therapies with mono- or polyclonal antibodies. The lymphopenia-induced homeostasis-driven proliferation leads to a novel homeostasis characterized by the expansion of cell subsets that characteristically show a phenotype of terminally differentiated cells such as CD8<sup>+</sup>CD57<sup>+</sup>CD28<sup>-</sup> T cells. Cells with these markers have long been linked with suppressor functions and recent data suggest their specific regulatory role. In addition cell phenotypes seen in transplant patients show striking similarities with changes in immune cells associated with aging. These phenotypic

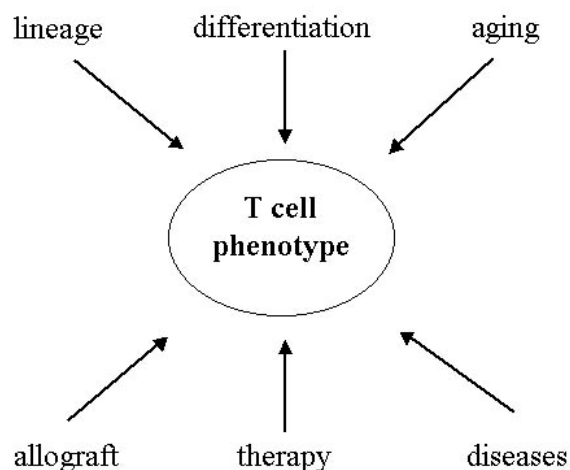
changes suggest an accelerated aging of the immune system in transplantation.

### 2. INTRODUCTION

Fluorescence-based flow cytometric analysis has become a widely used diagnostic tool to analyze phenotypes of cells. The underlying review gives a survey of changes in immune phenotypes associated with immunosuppressive therapy in transplant patients.

The phenotype of cells is defined by the expression of hundreds of unique surface proteins, glycoproteins, and glycolipids on the membrane. These molecules distinguish cell lineages and stages of their differentiation. Phenotyping cells started with the development of antisera, such as anti-Ly-1 and anti-Ly-2, separating different subsets of lymphocytes. The development of monoclonal antibody technology was

## Phenotypic changes with immunosuppression



**Figure 1.** Components that induce changes in the phenotype of T cells.

instrumental in detecting individual cell surface molecules and identifying unique patterns of expression on different cell subsets (1-5).

The cluster of differentiation (CD) nomenclature was developed by the International Leukocyte Workshop to define this broad array of cell surface molecules. All monoclonal antibodies that react with the various epitopes of the same membrane molecule are grouped into an individual cluster. Practically all the genes encoding these CD molecules (syn. CD antigens or CD markers) have been cloned and the serologic epitopes have been identified. On the most recent Human Leukocyte Differentiation Antigen workshop (HLAD7 in Harrogate in the year 2000) about 80 new CD specificities were added. Now the CD index comprises more than 250 CD designations (for molecular, functional, and other data on the CD markers see the "Protein Reviews on the Web" PROW web site <http://www.ncbi.nlm.gov/prow/>) (6, 7).

The sheer number of CD molecules reflects the heterogeneity and complexity of cell populations. Cell lineages and subpopulations are traditionally differentiated by three-color flow cytometric analyses. But, due to the limited number of markers used, functionally and phenotypically distinct 'fine' cell subsets may not be detectable. Differences between surface molecules expressed and actually measured may explain some of the controversial and sometimes confusing data in regard to described changes in subsets (2, 3, 5, 8, 9). These methodological limitations inherent to flow cytometric data generated over the last 20 years have to be taken into account. Reflecting these considerations, the underlying review focuses more on general principles of phenotypic changes in 'broad' lymphocyte subsets than on patterns of expression to identify 'fine' cell subpopulations.

T cells play the key role in immune responses associated with both solid organ and bone marrow transplantation. T lymphocytes are the primary target of immunosuppressive therapy. Flow cytometry allowed for

the first time the routine analysis of T cell subsets both for diagnostic purposes and for therapeutic monitoring (1, 2). Analyses of T cell populations constitute the bulk of data on phenotypic changes associated with immunosuppression. Therefore primarily phenotypic changes in T cell subsets will be reviewed.

Figure 1 shows the key elements determining the T cell phenotype. Markers of cell lineage and state of differentiation primarily characterize the different T cell subsets. In general, phenotypic changes are due to aging, diseases, and therapies. The continuous presence of the allograft is an additional transplant specific factor. Characteristic phenotypes related to the normal T cell physiology and to the specifics of the transplant setting will be discussed in sections 3 and 4. Section 5 reviews the impact of immunosuppressive therapy on the expression of cell surface markers. Principal differences between immunosuppressive agents will be outlined and main mechanisms of action related to surface molecules will be discussed. The impact of these therapies on immune reconstitution reflected by cell surface molecule monitoring will be topic of section 6. The mechanism of regeneration and the establishment of a novel T cell homeostasis will be reviewed. The last point analyzes similarities in the phenotypic changes associated with transplantation and immunosuppressive therapy and accelerated aging.

### 3. PHYSIOLOGIC CHANGES IN IMMUNOPHENOTYPES

#### 3.1. Expression of cell surface markers during T cell development

Maturation describes the intra-thymic pathway of T cell development from progenitor cells to mature, single-positive CD4 or CD8 thymocytes. This process includes T cell receptor (TCR) gene rearrangement, positive and negative selection and is marked by characteristic changes in cell surface phenotypes.

In the early phase the progenitor cells do not express surface molecules characteristic of T cells. They are referred to as double-negative cells. The developmental progression of the double-negative population is marked by the expression of surface markers, particularly c-Kit, CD44, and CD25. Further maturation leads to the expression of a TCR and the progression to the CD4 and CD8 double-positive state. The early markers CD44 and CD25 are no longer expressed. Double-positive thymocytes that express the alpha-beta TCR-CD3 complex and survive thymic selection develop into either single-positive CD4 or single-positive CD8 thymocytes. This transformation is not well understood. One model postulates that interactions with class I MHC instructs the double-positive cells to differentiate into CD8<sup>+</sup> T cells and engagement with class II MHC selects for CD4<sup>+</sup> T cells, respectively. The intra-thymic maturation process takes about 3 weeks and around 98% of all thymocytes do not mature and undergo apoptotic cell death. The single positive thymocytes leave the thymus and enter the circulation as mature, naïve CD4<sup>+</sup> or CD8<sup>+</sup> T cells (2, 7). As described in section 6 extra-thymic maturation pathways exist and seem to mimic the thymic developmental steps.

## Phenotypic changes with immunosuppression

**Table 1.** Properties of CD8<sup>+</sup> T cells

	Naïve	Memory	Effector
<b>Surface markers</b>			
▪ CD45RA	+	-	+
▪ CD45R0	-	+	-
▪ CD11a	-/+	+	++
▪ CD11b	-	-	++
▪ CD27	+	+ <sup>1</sup>	-
▪ CD28	+	+ <sup>1</sup>	-
▪ CD49d	-	+	++
▪ CD57	-	-	++
▪ CD62L	+	- <sup>2</sup>	-
▪ CD95	-	++	+
▪ CCR7	+	- <sup>2</sup>	-
<b>Cytokines</b>			
▪ IL-2	+	++	-
▪ IL-4	-	+	-
▪ IFN-γ	-	++	++
▪ TNF-α	-	++	++
<b>Effector molecules</b>			
▪ CD95L (FasL)	-	-/+	++
▪ Perforin	-	-/+	++
▪ Granzyme B	-	-/+	++
<b>Functions</b>			
▪ Cytotoxicity	-	-/+	++
▪ Ag-induced proliferation	+	+++	-

Adapted from (3, 9, 19, 20), <sup>1</sup> Appay differentiates between early and late Ag-experienced memory cells, late memory cells are CD27<sup>-</sup> and CD28<sup>-2</sup> De Rosa describes memory cells as CD62L<sup>+</sup> and CCR7<sup>+</sup>

Post-thymic differentiation comprises the development of T cells from naïve to effector and memory cells as a result of antigen-driven proliferation. The general paradigm is that on exposure to specific antigen, naïve T cells get activated and proliferate rapidly into effector cells. A fraction of these activated cells survive and persist long-term as memory T cells (2, 7, 10-12). Naïve T cells are long-lived lymphocytes in a resting state, continuously circulating between blood and lymph system. They need secondary lymphoid tissue for their maintenance (13). The short-lived effector cells can enter non-lymphoid tissues and carry out specialized functions such as cytokine secretion, B cell help, and cytotoxic killing. CD4<sup>+</sup> effector T cells can be segregated into Th1 and Th2 cells according to the types of cytokines they produce (14). Memory T cells circulate in a resting stage, but on antigen-contact they expand more rapidly, with higher and more sustained proliferation peaks and broader responses than their naïve counterparts (13, 15-18). The differentiation into naïve, effector, and memory T cells is associated with characteristic changes in the expression of surface molecules, as shown in table 1 for the CD8<sup>+</sup> T cell subset (3, 19-23). However, no single surface marker is specific and phenotype per se is not function. Increasing the number of markers and the use of better functional tests improves specificity and identifies 'finer' subsets in cell populations (3, 24). A minimum of three differentiation markers is suggested to enumerate naïve T cells with better than 95% accuracy (9). Recently, subsets of CD4<sup>+</sup> T cells resembling

Th1 and Th2 cytokine patterns could be defined based on cell surface markers (25).

In addition to the antigen-dependent proliferation it is now well established that naïve T cells can also proliferate under lymphopenic conditions (10, 26-31). This homeostasis-driven proliferation (syn. lymphopenia-induced proliferation, homeostasis-driven memory T cell differentiation) of naïve T cells is not dependent on cognate antigenic stimulus but requires interaction of self-peptides plus MHC molecules. The antigen-independent T cell differentiation pathway is not associated with an up-regulation of early activation markers. But during homeostasis-driven proliferation the cells acquire the surface markers and functional properties of antigen-stimulated cells (10, 31). Thus, the 'memory' phenotype population consists of antigen-experienced and homeostasis-driven cells.

There is an on-going discussion about the stability of these phenotypic changes and the possibility of phenotypic reversion of memory cells back to naïve phenotype cells (32-35). Recent findings suggest that homeostatic memory T cells do not revert to naïve cells (10, 36, 37). The linearity of differentiation from naïve to effector to memory T cells is another area of controversy. There is no consensus whether a small proportion of effector cells eventually survive as memory cells or independent lines of development for effector versus memory cells exist (11, 18, 38-40).

The initial antigen encounter triggers differentiation of naïve cells. Recent studies show that cellular expansion, contraction, and longevity are tightly regulated. The initial antigen encounter results in at least eight to ten programmed cell divisions for the single cell. The antigen dose seems to determine size of the expansion (41-43). There is also evidence that the contraction phase is programmed, independent of the continued presence of antigen (44). The pattern seems to be similar for CD4<sup>+</sup> and CD8<sup>+</sup> T cells. But CD8<sup>+</sup> T cells divide more (15 divisions) and faster (6 hr per division) than CD4<sup>+</sup> T cells (9 divisions, 9-20 hrs per division). As a result, the magnitude of expansion and longevity is greater for CD8<sup>+</sup> T cells (45). Homeostasis-driven proliferation shows a comparable programmed cell differentiation. In comparison to antigen-driven proliferation the kinetics are slower (31, 37, 46).

### 3.2. Aging

Aging is associated with shifts in lymphocyte populations and changes in the expression profiles of T cell surface markers (for an excellent review see Pawelec et al (47)). The absolute numbers of peripheral lymphocytes remain rather constant throughout life (2, 48). The classical view of a generalized decline in humoral and cellular immune responses associated with aging ('immunosenescence') remains controversial as changes that include enhanced as well as diminished functions occur (47, 49).

The age-related changes in T cell differentiation and phenotypes are related to a large extent to the thymic involution. The output of T cells declines with age. This is

## Phenotypic changes with immunosuppression

associated with a shift from naïve to memory phenotype T cells and decreased double positive and increased double negative CD4 and CD8 cells in the peripheral circulation (49-53). Alternative sites and pathways of T cell development compensate in part the reduced thymic output (49, 54, 55). Overall thymic involution is a discontinuous process and the thymus continues to generate T cells through age (52, 53). The ratio of CD4<sup>+</sup> to CD8<sup>+</sup> T cells does not show a marked change with age. Of 24 published reports, half did not demonstrate any age-related change, 6 reported small increases and 6 reported decreases in the ratio (2). T cells show an age-associated increase in the expression of NK cell markers and a down-regulation of the co-stimulatory molecule CD28 (56, 57).

Due to the age-related impact on the distributions of lymphocyte populations and on surface markers like CD28 or CD57 it is mandatory to use age-matched controls when analyzing phenotypic changes.

### 3.3. Lymphocyte compartments

The analysis of lymphocyte subset data has to take into account biological variability, life spans, and provided diagnostic window.

Seasonal and monthly changes have been described for total lymphocyte counts and CD4<sup>+</sup> and CD8<sup>+</sup> T cells (58). Marked diurnal rhythms occur and blood samples for serial lymphocyte subset analyses should ideally be taken at a standard time. Changes in the absolute CD4 counts up to 60% have been observed (59-61). Overall intra-individual levels of absolute lymphocyte counts, CD4<sup>+</sup> and CD8<sup>+</sup> T cells are stable (59, 62).

Peripheral blood contains only 2 to 3% of the total lymphocyte pool. In addition, the various lymphocyte subsets show different recirculation patterns and migrate into different sites. It is estimated that an individual naïve T cell circulates from the blood to the lymphoid tissue and back again as often as 1 to 2 times per day. The average transit time of lymphocytes in the blood is estimated to be 30 minutes (7, 63). The heterogeneity in the circulation and homing behavior is reflected in the differences in expression of surface molecules between the different T cells populations (7, 64).

The life spans of different lymphocyte subsets are still not exactly known. McLean and Michie investigated in vivo proliferation rates. They calculated that naïve lymphocytes divide once every 3.5 years and memory lymphocytes once every 22 weeks. Overall T cell death rates were very small (65). Recently various new models to study lymphocyte kinetics were proposed (66, 67). The heterogeneity of the lymphocyte subpopulations, differences in life spans, varying recirculation patterns, and different compartments make the analysis of phenotypic changes in peripheral lymphocytes difficult. Peripheral blood subset analysis permits only a very limited view of the immune system (63, 67).

## 4. SPECIFICS OF THE TRANSPLANT SETTING

Apart from the immunosuppressive therapy, the immune responses caused by allograft and/or infections are

major factors, which induce phenotypic changes in lymphocyte surface markers in the transplant setting. Flow cytometry of lymphocyte subsets has been used in transplant medicine for both monitoring and diagnostic purposes since the early eighties (4, 68, 69).

Data on the diagnostic value of peripheral lymphocyte subset measurements in the differential diagnosis of acute rejection versus infection are controversial. Most studies analyzed changes in the ratio of CD4<sup>+</sup> to CD8<sup>+</sup> T cells in regard to the occurrence of rejections or infections. Increases in the ratio seem to correlate with acute rejection and relative decreases with viral infection (4, 68-72). However, these changes are not specific enough. Additional effects, in particular of the immunosuppressive therapy, have a major impact on the profiles (73-78). Monitoring of activation markers like CD25 or CD71 has been recommended for the differential diagnosis between rejection, infection, and drug nephrotoxicity (4, 79). Recently, CD28 expression was reported to be associated with acute rejection in liver transplants (80). But overall, all these markers are not routinely used in transplant monitoring (4, 81).

The measurement of HLA-DR expression on monocytes is an interesting approach to assess the risk of over-immunosuppression. The expression was significantly lower in patients who developed bacterial sepsis. In addition it could be shown that the down-regulation was related to the dose of prednisolone given (82, 83).

The pre-transplant status of the patients may be another factor influencing distribution and numbers of lymphocyte populations. Hemodialysis patients have a decrease in their cellular immunity, which is in part reflected by lower absolute T cell numbers and changes in the subsets (84, 85). Operative trauma as well as non-immunosuppressive therapy may influence lymphocyte numbers. Immediately after surgery, decreases in T cell numbers of up to 60% have been reported (76, 77). Heparin therapy, for example, is known to cause an acute decrease in circulating lymphocyte counts and subsets (86). The underlying disease in the transplant recipient may also have an impact on lymphocyte subsets. In particular autoimmune diseases show marked perturbations in lymphocyte phenotypes (59).

Overall, these changes in lymphocyte phenotypes related to the transplant setting have to be taken into account when analyzing the impact of the immunosuppressive therapy.

## 5. IMMUNOSUPPRESSIVE THERAPY

Surface molecules serve two key strategies in regard to immunosuppressive therapy. First, they are used as biomarkers in pharmacodynamic assays (87-91). Second, surface receptors and ligands on cell membranes are key targets for immunosuppressive agents.

The currently used immunosuppressive drugs can be divided into two classes according to their main site of

**Table 2.** Currently used immunosuppressive drugs

Small molecule drugs	Protein based drugs
Immunophilin binding drugs: <ul style="list-style-type: none"> <li>▪ Cyclosporine - Sandimmune®, Neoral®</li> <li>▪ Tacrolimus - Prograf®</li> <li>▪ Sirolimus - Rapamune®</li> </ul>	Polyclonal T cell antibodies: Atgam®, ATG-Fresenius®, Thymoglobulin® Monoclonal antibodies: Anti CD25: Daclizumab - Zenapax® Basiliximab - Simulect® Anti CD3: Orthoclone OKT3® Anti-CD52: Alemtuzumab - Campath-1H®
Purine/pyrimidine synthesis inhibitors: <ul style="list-style-type: none"> <li>▪ Mycophenolate Mofetil - CellCept®</li> <li>▪ Azathioprine - Imuran®</li> </ul>	
Corticosteroids	

action (see Table 2). Small molecule drugs act primarily on intracellular pathways and are predominantly used in maintenance therapy. Protein-based drugs act primarily extracellularly by directly targeting cell surface molecules. They are used in induction and rejection therapies (92-95).

### 5.1. Maintenance therapy

In maintenance therapy, the small molecule drugs are usually used in combination. It is therefore difficult to relate changes seen in surface molecules to an individual drug. In addition, the effects on surface marker expression are less pronounced than those achieved with the biological agents. However, some phenotypic changes in immune cells have been associated with special drug therapies as outlined in the following.

Calcineurin inhibition by cyclosporine or tacrolimus influence intra-thymic T cell development. In particular, it inhibits the progression of double-positive to single-positive thymocytes, leading to a reduction in mature T cells (96-102). In addition, a disruption in the process of negative thymic selection seems to lead to an increase in self-reactive T cells (101). The sequential analysis of lymphocyte subsets in relation to the combined treatment with cyclosporine, azathioprine, and either anti-CD3 or polyclonal anti-T cell antibodies did not provide a characteristic pattern and supported the overall variable effect of these drugs on T cell subsets (103). Two interesting studies looked at changes in lymphocyte populations in children from renal transplant mothers, who were exposed to cyclosporine, azathioprine, and steroids in utero. Both studies described only minimal effects on T cell numbers and functions (104, 105). However, findings on B cells were contradictory. Takahashi et al described a severe B cell depletion in the newborns and concluded a high sensitivity of the B cell line to immunosuppressive therapy in utero.

Azathioprine has one of the earliest agents used in transplant medicine. Its leukopenic effect is well known. Alamartine and co-workers showed a particular strong influence of azathioprine on number and function of NK cells (106). One study looked at phenotypic changes in CD4<sup>+</sup> T cells of patients with myasthenia gravis who were treated with thymectomy and low-dose immunosuppression of steroids or azathioprine. Overall, they did not see any significant changes to a group of healthy controls (107). It is of interest that thymectomy per se does not seem to lead to phenotypic alterations.

The inosine monophosphate dehydrogenase inhibitor mycophenolate mofetil (MMF) has an impact on

the expression of T cell surface molecules. It inhibits the expression of receptors (for IL-2, transferrin, and TNF- $\alpha$ ), of adhesion molecules (LFA-1 and ICAM-1), and of co-stimulatory molecules (CD154 and CD28) (87, 108). In addition, an inhibitory function on antibody formation and the induction of apoptosis of activated peripheral T cells have been described (109, 110).

Glucocorticoids are used in maintenance and rejection therapy. They induce a dose-dependent but short-lived acute lymphopenia and have a profound effect on thymocytes. Both CD4 and CD8 counts drop with a return to normal values within 48 hours (59, 111, 112).

### 5.2. Induction and anti-rejection therapy

The protein-based drugs are primarily used in induction and/or rejection therapy. They are directed against receptor-ligand targets located on the surface of the cell membrane. These drugs can be divided into monoclonal and polyclonal antibodies (see table 2).

Each monoclonal antibody preparation acts specifically on one single surface marker. OKT3® was the first anti-T cell monoclonal antibody available for clinical use (68). Now various monoclonal antibodies against different types of surface molecules are available. Targets include the T cell receptor (TCR/CD3) complex and its CD4 and CD8 co-receptors, co-stimulatory molecules, cytokine receptors, and adhesion molecules. According to their effect depleting and non-depleting monoclonal antibodies are distinguished (2, 113).

The polyclonal antibody preparations contain a mixture of antibodies against a broad variety of surface antigens. They comprise both depleting and non-depleting effects. More than 20 different antibody specificities have been described (114-119). Starzl and his group first introduced polyclonal antilymphocyte sera into clinical protocols (120).

#### 5.2.1. Effects on surface molecules by non-depleting agents

In addition to depletion, the major immunosuppressive effects of antibodies are achieved by modulation, coating, and activation of surface molecules.

Modulation is the most relevant non-depleting, functional effect. It describes the reversible decrease in the expression of cell surface molecules due to cross-linking between antibody and surface antigen. The antibody-antigen complex is rapidly internalized and not re-

## Phenotypic changes with immunosuppression

expressed, as long as the antibody is present. Modulation is antigen-specific, dose-dependent, rapid and not altered by other immunosuppressive drugs, such as corticosteroids or calcineurin inhibitors. Revillard and his co-workers have extensively investigated the modulation of surface antigens. They developed a method using indirect immunofluorescence to measure precisely modulation by FACS analysis (121, 122).

The monoclonal antibody OKT3® exerts part of its action due to the reversible down-regulation of the TCR/CD3 complex. This modulation is associated with T cell unresponsiveness to antigens or allogeneic cells. The molecules are re-expressed within a few hours when OKT3® serum levels decrease because of immunization or cessation of treatment (123-126).

The polyclonal antibody preparations induce the modulation of a number of surface markers. Down-regulation of molecules controlling T cell activation, including the TCR/CD3 complex, CD2, CD4, CD8, CD5, CD6, and regulating leukocyte-endothelium interactions, including LFA-1 (CD11a/CD18), ICAM-1 (CD54) have been shown for Thymoglobulin®. This effect occurred within 2 hours and was seen for up to 4 weeks after treatment (116, 121, 127). Similar effects have been described for other polyclonal T cell antibodies (114, 117, 118, 128). Recently, Pistillo et al detected anti-CTLA4 antibodies in polyclonal antibody preparations (119).

Antibody preparations might also act by 'blindfolding', i.e. binding or coating of surface molecules without causing modulation. This can also inhibit cell function by a variety of nonexclusive mechanisms like denial of appropriate co-stimulation, delivery of negative signals, and reduction of cellular adhesion (114, 129).

The recently introduced humanized monoclonal antibodies Basiliximab (Simulect®) and Daclizumab (Zenapax®) act on activated T cells by binding to the alpha chain (CD25) of the IL-2 receptor. This blocks T cell activation and proliferation. The duration of this blockade is dose-dependent. Using the clinically recommended dosages CD25 expression on peripheral lymphocytes is absent for about 20 to 45 days (130-133). The IL-2 receptor antagonists do not induce any lymphocyte depletion or manifest changes in lymphocyte subsets (130, 132). However, in vitro studies demonstrated some degree of antibody-dependent cell cytotoxicity (ADCC) associated with anti-IL-2 receptor antibodies (134).

Binding to various surface molecules has been shown for the polyclonal antibodies in several studies (115-118, 127, 128, 135). It is of interest, that this binding is not cell lineage specific and might be due to cross reactivity of epitopes on different cell lines. The polyclonal preparations Atgam® or Thymoglobulin® bind preferentially to T cells. However low level binding to NK cells, B cells, monocytes, granulocytes, erythrocytes, and platelets has been demonstrated (121, 122, 127, 135). A transient fall in the non-lymphoid cell lines might be due to this non-lineage specific binding and is usually seen when high

doses of the polyclonal antibodies are used. Probably due to the high turn over rates of these cells the observed decreases in cell numbers are short-lived.

Both binding and modulation of specific surface molecules lead to phenotypic changes. The specific fluorescent antibodies used for the detection of the related surface molecules cannot bind. Thus, numbers of peripheral T cells based on flow cytometry measurements may be misleading and false low. In case of monoclonal or polyclonal anti-T cell therapies, up to 30 to 40% of circulating lymphocytes lack T and B cell markers. This population of so-called null cells is usually not detected by flow cytometry (121).

Engagement of antibodies with the receptors or ligands might lead to activation of the target cells. Activation of T cells has been demonstrated in particular for the monoclonal antibodies anti-CD3 (OKT3®) and anti-CD52 (Campath-1H®) and to a lesser degree for polyclonal anti-T cell preparations (121, 123, 124, 128, 136). This T cell activation is associated with up-regulation of CD25 and the synthesis of pro-inflammatory cytokines and the so-called "first dose syndrome". The mitogenic effect on lymphocytes varies between the different antibody preparations and is dose-dependent. Bonnefoy-Berard could show that Thymoglobulin induces in addition to T cell activation, a transient B cell activation. But unlike OKT3 it did not support B cell proliferation and differentiation into immunoglobulin-secreting cells. At low concentrations Thymoglobulin induced an incomplete T cell activation in vitro (121, 124). Merion showed that the polyclonal antibody Atgam® induced a partial T cell activation associated with marked increases of CD28 and IL-2 receptor (CD25) in the absence of proliferation and leading to anergy (128).

The functional effects of modulation, binding, coating, and activation are related to the presence of antibodies and therefore determined by the pharmacokinetics of the individual antibody preparation. In contrast to these rather temporary effects, cell depletion induces profound short- and long-term changes.

### 5.2.2. Depletion of target cells

The depletion of target cells is probably the most potent immunosuppressive effect. Surface molecules serve as targets for monoclonal and polyclonal antibodies. The binding leads to the elimination of these target cell lines from the blood compartment. Information on penetration, mechanisms of action, and degree of depletion of these drugs in lymphoid and graft tissues is still needed.

#### 5.2.2.1. Mode of action

In general, depleting monoclonal or polyclonal antibodies lead to a rapid (within 2 hrs) elimination of targeted cells from the peripheral blood compartment by complement- or Fc-dependent lysis and/or sequestration into the lymphoid tissues. Complement-dependent cell lysis (CDC) is the major mechanism of depletion used by the monoclonal antibody CD52 (Campath-1H®). The administration of anti-CD52 leads to a rapid and profound

## Phenotypic changes with immunosuppression

drop in the circulating cell numbers of B and T cells, monocytes, macrophages, and eosinophils. This is due to the high density of the surface molecule CD52 (comprising about 5% of the lymphocyte cell surface) and its broad distribution on these cell lines (137, 138). Complement-dependent cell lysis is also a major mechanism for T cell depletion induced by polyclonal T cell antibodies. At therapeutic doses the extent of depletion is lower than with the anti-CD52 monoclonal antibody, due to the lower density of the targeted surface molecules. The monoclonal antibodies OKT3® and anti-CD25 (Basiliximab® and Zenapax®) do not induce complement-dependent cell lysis (123, 131, 134). In addition, complement-dependent actions do not play a major role in the extra-vascular space, as tissue concentrations of complement are low.

T cell activation inducing apoptotic cell death is an additional mechanism of cell depletion described for the monoclonal antibody OKT3® and the polyclonal antibodies (123, 127, 139). The apoptosis of T cells is not mediated by death receptors and not inhibited by steroids, calcineurin inhibitors or rapamycin. This mechanism is characterized by rapid kinetics (< 24 hours) and has been found to be the main mode of action of depletion induced by Thymoglobulin® in secondary lymphoid tissues (127). Cell margination due to the up-regulation of adhesion molecules as well as sequestration and opsonization in the reticulo-endothelial system explains the rapid fall in peripheral T cell numbers induced by OKT3® (123, 140).

Revillard and his group could demonstrate further in vitro mechanisms accounting for the selective depletion of activated T cells. Low sub-mitogenic levels of Thymoglobulin induced the expression of TNF-alpha and CD95 (Fas) on resting T cells. These cells mediate via Fas/Fas-Ligand interaction, the apoptotic cell death of activated T lymphocytes. Steroids and calcineurin inhibitors blocked this selective action. They could also show that antibody-dependent cellular cytotoxicity is a second mechanism depleting selectively activated T cells (139, 141).

### 5.2.2.2. Impact on cell subsets

The various anti-T cell antibody preparations induce basically comparable changes in the lymphocyte subsets. The earliest studies described the general pattern of a rapid (within 2-3 days), profound (lymphocyte levels < 10% or <300 cells per microliter compared to pre-treatment levels), and sustained (weeks to months after cessation of therapy) lymphopenia induced by the anti-lymphocyte sera (120, 142-144). The overall degree and duration of lymphocyte depletion is higher with the polyclonal antibody preparations than with OKT3® (145-148). The drop in the T cell counts is most pronounced with Thymoglobulin® in comparison with the various polyclonal preparations (135, 149-151). Changes in the specific T cell subsets as well as other cell lines are not that consistent. Zimmermann described a dose dependent effect of anti-T cell sera. Low doses eliminated primarily T helper cells while sparing other subsets (152). A marked decrease in NK cells (CD16+CD57-) associated with rabbit polyclonal antibodies but not with OKT3® is described by

Zaltzman (146). B cell counts dropped slightly during therapies with rabbit anti-T cell globulins (148, 153). Bonnefoy-Berard demonstrated the induction of apoptotic cell death of activated B cell lines by in vitro experiments (154, 155). All studies showed a quicker recovery of the subset of CD8<sup>+</sup> T cells in comparison to the CD4<sup>+</sup> T cell subset (145-148, 151, 156, 157). It is of interest that an immunotoxin directed against CD3 and first investigated by Knechtle and his group in non-human primates showed the same pattern of depletion and recovery of CD4<sup>+</sup> versus CD8<sup>+</sup> T cells (158). The monoclonal antibody against the CD52 surface molecule (Campath-1H®) shows the most pronounced effects. A profound lymphopenia, decrease in monocytes and macrophages, and often platelets is induced by the administration of Campath-1H® (137, 159, 160). Again the recovery of CD4<sup>+</sup> T cells is far slower than that of the CD8<sup>+</sup> subset.

### 5.2.2.3. T cell monitoring and dose-adjustment

The absence of T cells from the circulation is seen as a biomarker for monitoring the efficacy of therapies using depletion as main immunosuppressive action. There are a number of studies suggesting the effectiveness of T cell monitoring to achieve potent immunosuppression without over-immunosuppression.

In particular Clark and Shenton showed that T cell monitoring provides a means for dose-adjustment of polyclonal antibodies. The daily dose of the anti-lymphocyte agent was adjusted to achieve a target level of 50 CD3<sup>+</sup> T cells per microliter. This regimen allowed a reduction in the total drug dose with no loss of efficacy but concomitant reduction in serious viral infections plus a cost benefit (161, 162). Safety, efficacy, and cost benefit of this approach have been demonstrated in several studies (163-171). However, the optimum target level of cell numbers is still not clear and there still remains the question of whether measurements of total lymphocyte counts are sufficient or CD3<sup>+</sup> measurements are needed (165, 166, 168). This dilemma is partly a result of methodological differences such as the high inter-laboratory variability, the lack of standardization, and the problem of reproducibility of cell numbers in the severely lymphopenic blood counts (8, 162).

## 6. IMMUNE RECONSTITUTION AND LONG-TERM CHANGES

Early phenotypic changes occurring during the use of immunosuppressive agents directed against surface molecules were described in section 5. However, a prospective study also showed profound long-term effects on T cell populations after short-term T cell depletion in renal transplant patients (147, 172). Similar findings have been reported for various disease entities in which T cell depleting therapies are used (33, 158, 159, 173-176). Extent, kinetics, and functional implications of these changes are of major importance for the 'integrity' of the immune system and its responses to self and foreign antigens. In contrast to short-term blocking agents the functional impairment attained with depleting strategies might be sufficiently long enough to put adequate regulation in place in the setting of transplantation (160).



## Phenotypic changes with immunosuppression

These long-term phenotypic changes and emerging principles of immune reconstitution are discussed in the following sections. Because few studies analyze long-term changes in lymphocyte subsets after depleting therapies in solid organ transplantation, data on cell depletion in other disease entities, such as bone marrow transplantation, malignancies or autoimmune diseases are included.

### 6.1. Regeneration and novel homeostasis

#### 6.1.1. Homeostasis-driven proliferation

In an adult mammal the total number of lymphocytes remains constant. Changes in total counts unmask a tendency of this cell type, to approach the previous stationary distribution of population densities, i.e. homeostasis (177, 178). T cell depleting therapies generate pronounced changes in lymphocyte populations. Recently it has been shown in mice that lymphopenia induces the expansion of the residual T cells. During this so-called homeostasis-driven proliferation, the naïve T cells acquire the cell surface markers and functional properties of antigen-induced memory T cells (27, 29, 179). Despite the phenotypic similarities in the memory cells there are significant differences between antigen- and homeostasis-driven proliferation pathways. In particular, the latter shows a slower rate of expansion, the proliferating cells in lymphopenic hosts do not increase significantly in size or become overtly activated to express CD69 and CD25 and they do not acquire effector cell functions as do antigen-stimulated T cells (31, 37, 46). Recent work strongly suggests that the homeostasis-driven proliferation restores only the memory T cell compartment. The reconstitution of the naïve T cell compartment seems to depend on de novo T cell development in the thymus (21, 31, 180).

The phenotypic changes seen in transplant patients after T cell depletion fit very well within the concept of the homeostasis-driven memory T cell proliferation. 'Surviving' residual naïve and memory T cells undergo proliferation to fill the empty space. This homeostasis-driven memory T cell proliferation leads to a further loss of naïve T cells and an increase of T cells with a memory phenotype (173, 174, 181). It explains the marked over-representation of memory-phenotype cells in adult transplant patients following T cell depletion. The extent of naïve T cell regeneration depends on the 'thymic' age. A recent study demonstrated a significant inverse relation between age and reconstitution of naïve T cells in women who received high dose chemotherapy for breast cancer (182). The extent of extra-thymic generation of naïve CD8<sup>+</sup> T cells is controversial. The study after high dose chemotherapy showed a similar reconstitution pattern for naïve CD4<sup>+</sup> and naïve CD8<sup>+</sup> T cells (182). By contrast, in a thymectomized patient, the generation of CD8<sup>+</sup> T cells with a naïve phenotype (CD45RA<sup>+</sup>) was demonstrated, but this patient could not generate naïve CD4<sup>+</sup> T cells (181, 183).

The most striking finding on phenotypic monitoring after T cell depletion is the occurrence and persistence of a pronounced inversion in the ratio of CD4<sup>+</sup> to CD8<sup>+</sup> T cells (147, 172-174, 181-185). This inversion

reflects differences in the regeneration pathways. Reconstitution of CD4<sup>+</sup> T cells is dependent on the thymus, whereas CD8<sup>+</sup> T cells can be restored by extra-thymic maturation (33, 186-188). This explains the age-dependency of the regeneration of CD4<sup>+</sup> T cells and the changes in the ratio of CD4<sup>+</sup> to CD8<sup>+</sup> T cells.

In addition, homeostasis-driven proliferation requires the engagement of the T cell receptor (TCR) with MHC-molecules loaded with self-peptides plus other stimulating factors, in particular cytokines. This TCR-mediated proliferation is the same as for antigen-induced T cell proliferation, except in the latter case the peptides are derived from exogenous antigens. The encounter of the TCR with MHC-class II determines the proliferation of CD4<sup>+</sup> T cells, MHC-class I engagement leads to CD8<sup>+</sup> T cells (31, 46, 179, 189-192). This selection of cell lineages mimics the intra-thymic maturation pathway. The overall higher quantity of MHC class I expression in the periphery and the potentially higher antigenic load in the immunocompromised transplant patient might be an additional explanation for the dominant expansion of CD8<sup>+</sup> T cells relative to the CD4<sup>+</sup> T cells.

In addition to the observed CD8<sup>+</sup> T cell expansion the described mechanisms of depletion-induced T cell proliferation might explain another clinical phenomenon; i.e. the association between cell depletion and autoimmune phenomena (193-196). In lymphopenic mice only about 30 percent of the residual T cells undergo homeostasis-driven proliferation (21, 29). Cells that preferentially expand are those with a high affinity for self-peptide/MHC and those recognizing the most abundant self-peptide/MHC complexes (10, 12, 46, 190). The expansion of the most auto-reactive T cells might be one explanation for the organ-specific autoimmune diseases described in mice after thymectomy and/or irradiation or recently in patients with multiple sclerosis who received anti-CD52 antibodies (194, 195). However, in regard to the multitude of patients being treated with T cell depletion and the very low incidence of clinically manifest autoimmune diseases there must be additional regulatory mechanisms, which are not yet well understood. In this respect it is of interest, that one major characteristic of homeostasis-driven T cell differentiation (comparable to the intra-thymic T cell maturation and differentiation) is the absence of inflammation. This is a major difference from antigen-driven T cell differentiation.

#### 6.1.2. Population kinetics and homeostasis

In general, each lymphocyte population demonstrates unique regeneration kinetics. In addition, both numbers and distribution of the lymphocyte subsets reached in the new steady state differ from pre-treatment levels, healthy control numbers, and levels shown by transplant patients who did not receive T cell depleting therapies. However, these observations are based on a small number of studies on repopulation patterns of lymphocyte subsets in adult patients treated with depleting antibodies for different disease entities. The groups of Bas and Mueller measured lymphocyte subsets in renal transplantation, Davison, Novitzky, and Heitger in bone



marrow transplantation, Brett and Jendro in rheumatoid arthritis, and Mackall and Fagnoni in malignancies (33, 147, 148, 172-175, 181-184, 188, 197, 198). Therefore, the patterns described below reflect general trends, highlighting clearly, the need for further detailed studies.

### 6.1.2.1. Dynamics

The most profound and persistent changes are seen following cell depletion with the anti-CD52 monoclonal antibody Campath-1H® (137, 160). Macrophages, NK cells, and B cells typically recover within 1 to 3 months. Total lymphocyte and T cell numbers remain below normal for up to two years (174, 175, 184). Following polyclonal antibodies or chemotherapy it takes about 12 to 24 months to reach normal total lymphocyte and T cell counts. CD8<sup>+</sup> T cells recover rapidly within the first 3 to 6 months and often maintain higher steady state levels than before treatment. In contrast, CD4<sup>+</sup> T cells show an age-dependent very slow recovery and remain below normal levels for as long as adult patients have been followed up (10 years) (33, 55, 147, 172-175, 182, 183, 199). The recovery of the naïve T cell population is age-dependent, as discussed above. In adult patients the naïve T cell numbers stay below the normal range for at least 12 to 18 months. The naïve CD8<sup>+</sup> T cells seem to recover quicker than the naïve CD4<sup>+</sup> T cells. The memory compartment recovers within 3 months and stays static throughout the follow-up (174, 181, 182).

### 6.1.2.2. Plateau, niches, and regulation

The time point of a new steady state seems to be determined by the total T cell numbers. Once they have normalized, i.e. after 12 to 24 months, numbers and distribution of the CD4<sup>+</sup> and CD8<sup>+</sup> population remain stable. This is best seen in the persistence of the depressed ratio of CD4<sup>+</sup> to CD8<sup>+</sup> T cells (147, 148, 172, 174, 182, 199, 200). This is a surprising finding. One would expect a progressive increase in the CD4<sup>+</sup> T cell population and parallel decrease in the CD8<sup>+</sup> T cells to eventually reach pre-depletion levels. However, data from the group of Rocha and Freitas obtained in murine experimental models support the observation of a development of new plateaus for the individual populations, independent of the levels before depletion (191, 201-204). The so-called 'Red Queen Hypothesis' summarizes the population kinetics: "You need all the running you can do to stand still" (202, 205). Once a novel homeostasis has been established the total sizes of the CD4<sup>+</sup> and CD8<sup>+</sup> populations remain stable, quantitative changes occur only within the two compartments. In this theorem, the total number of T cells is determined by the availability of space and resources. As the CD4<sup>+</sup> and CD8<sup>+</sup> populations harbor the same ecological niche, they are closely linked, both during the regeneration period and the new steady state (178, 191, 204). Homeostatic proliferation and 'Red Queen Hypothesis' nicely explain the general patterns in the expansion of the CD8<sup>+</sup> and restriction of the CD4<sup>+</sup> populations during the regeneration period and the persistence of the ratio of CD4<sup>+</sup> to CD8<sup>+</sup> T cells once the new homeostasis has been established.

The memory and naïve T cell populations show a different pattern. The population of memory T cells

recovers rapidly and stays static throughout the period of observation. In contrast, the naïve T cell population recovers very slowly and keeps increasing in numbers independent of the memory population (182, 183). The accumulation of memory cells will not lead to the contraction of the naïve T cell pool. Again supported by experimental data in mice these two populations seem to be independently regulated (31, 191, 192, 204). This might reflect the biological necessity to have a sufficient memory population for recall responses against known antigens and to be prepared for new antigens with a diverse and broad population of naïve T cells.

In addition to these population dynamics, mice data suggest an independent regulation for the B and T cell compartments, resting and activated B cells, and for the T cells with alpha/beta and with gamma/delta receptors (191).

### 6.1.2.3. Functional implications

The described kinetics and mechanisms are primarily based on changes in lymphocyte subsets defined by phenotypic markers. This review cannot cover the multiple biological implications. Loss and slow recovery of naïve T cells and increase in cells with a memory phenotype is associated with a marked loss in diversity and a restriction in the TCR repertoire (46, 182, 197, 206, 207). Immunizing strategies have to account for the slow recovery of naïve T cells and immunization is probably not effective within the first 12 months of reconstitution. During the period of reconstitution the susceptibility to infections is higher. Further, since T cell regeneration is highly age-dependent, depleting therapies have to take the patient's age into account. The homeostasis-driven proliferation and cell maintenance is both dependent on space, competing populations and resources. Cytokines play a crucial role (189, 208, 209). The therapeutic application of specific cytokines might be one approach to influence the recovery rates and distributions of various populations. Depletion and regeneration seem to support the generation of auto-reactive cells. On the other hand the replacement of the resident perinatal lymphocyte compartment by the adult cells is believed to be the basis for its increased susceptibility to tolerance induction (210, 211). It is of interest that this period of reconstitution and the establishment of a novel homeostasis with the expansion of specific cell subsets reflect the concept of separating the post-transplant course into a period of adaptation and a period of post-adaptation (160, 212). Adaptation processes seem to predominate during the first 3 to 6 post-transplant months. During this time a sufficient maintenance immunosuppressive therapy is needed. These months of increased immunity against the allograft are followed by the so-called post-adaptation period, during which the likelihood of acute rejection episodes is very low and a significant reduction in immunosuppression is possible.

However, the process of homeostasis-driven lymphocyte differentiation, the overlap with antigen-induced proliferation, the role of environment, niche segregations, antigen/allograft persistence in inducing effector functions or anergy/tolerance are still far from clear.

### 6.2. Expansion of specific cell subsets

Phenotypic monitoring in transplant patients does not only show changes in the major lymphocyte subsets. In our long-term study in renal transplant patients, the increase in the CD8<sup>+</sup> T cells was characterized by a progressive expansion in T cells co-expressing CD8 and CD57 (147, 172). This increase was seen in all transplant patients, irrespective of the initial therapy. However, patients treated with T cell depletion had the most pronounced increases. About 70 to 80% of all CD8<sup>+</sup> T cells expressed CD57. Other groups have reported similar expansions of this cell subset in both solid organ and bone marrow transplantation (32, 34, 35, 213-216). In healthy subjects, CD8<sup>+</sup>CD57<sup>+</sup> T cells are a small subset, comprising about 1-15% of the peripheral blood lymphocytes. The glycoprotein CD57 (syn. Leu-7, recognized by the monoclonal antibody HNK-1) is primarily expressed on NK cells. In addition, it is present on a fraction of CD3<sup>+</sup>CD8<sup>+</sup> cells and a small number of CD3<sup>+</sup>CD4<sup>+</sup> cells (217). Increases of CD8<sup>+</sup>CD57<sup>+</sup> T cells have been primarily associated with human cytomegalovirus (CMV) infections (217-224). In a longitudinal study Dessaint and his group analyzed the kinetics of phenotypic changes in lymphocyte subsets in allograft recipients with primary CMV infections (221). CMV viremia was associated with a lymphocytosis due to elevated CD8<sup>+</sup>CD57<sup>+</sup> T cells and phenotypic signs of T cell activation. When viral cultures became negative the activation markers disappeared. During the second to sixth month the subset of CD8<sup>+</sup>CD57<sup>+</sup> cells was replaced by CD8<sup>+</sup>CD57<sup>-</sup> cells (221). The CD8<sup>+</sup>CD57<sup>+</sup> cells became the predominant T cell population and their persistence for several years strongly correlated with the expansion of CD8<sup>+</sup> cells. In a following study they analyzed the recall response to CMV (224). Again, the accumulation of CD8<sup>+</sup>CD57<sup>+</sup> T cells was delayed, progressing over a period between 2 and 8 weeks after the onset of CD8 lymphocytosis. This delay in the occurrence of the CD8<sup>+</sup>CD57<sup>+</sup> T cells was also reported in a study in cardiac transplant patients. The CD57<sup>+</sup> T cells expanded 6 to 8 weeks after the start of the CMV infection (225). These findings suggest a crucial role of CMV in the development of this specific cell subset.

However, it is not clear whether increases in CD8<sup>+</sup>CD57<sup>+</sup> T cells are exclusively seen in association with CMV infections. Expansions of this specific cell subset have been reported in diseases such as rheumatoid arthritis (226, 227), Crohn's disease (228), or HIV (229, 230). These diseases are comparable to the transplant situation. They are characterized by chronic inflammation/immune activation and also by an increased incidence of CMV infections. An expansion of CD57<sup>+</sup> T cells may occur in the absence of clinical symptoms of CMV infection (221-223). It has been shown, that patients without detectable CMV viremia may have a persistent IgM response against specific CMV epitopes lasting over years (231, 232). The CD8 expression implies a MHC class I restriction for pathogens of intracellular origin. Thus, the persistent and ubiquitous CMV might induce by repeated antigenic stimulation and/or MHC-class I up-regulation an environment for the expansion of CD8<sup>+</sup>CD57<sup>+</sup> T cells. On

the other hand, the induction of the CD8<sup>+</sup>CD57<sup>+</sup> T cell population is slower than the development of CMV-directed humoral and cellular immune responses (221, 224, 225). Treatment- or disease-induced T cell depletion is associated with the strongest expansion in the CD8<sup>+</sup>CD57<sup>+</sup> T cell subset (147, 182, 223, 229, 230). The differences in the sizes of the CD8<sup>+</sup>CD57<sup>+</sup> subsets between depleting and non-depleting therapies were not associated with different incidences in CMV infections or markers of cellular immune activation (147, 172). Therefore, the expansion of CD8<sup>+</sup>CD57<sup>+</sup> T cells might be a characteristic of homeostasis-driven proliferation, in an environment with a high probability of CMV-related antigenic stimulation.

The functional role of the CD8<sup>+</sup>CD57<sup>+</sup> T cells is a focus of intense research. It has been shown, that the CD8<sup>+</sup>CD57<sup>+</sup> subset is clonally derived. CD8<sup>+</sup> T cells in the CD57 compartment express a highly restricted TCR repertoire (32, 217, 233). Cells with CD57 expression are terminally differentiated cytotoxic effector cells. Because of their specific biology and restricted repertoire they cannot be considered effective for either novel nor recall antigens. They may be important in the immunosuppression associated with CMV infection (217, 233-235). Contact-dependent suppression of CD8<sup>+</sup>CD57<sup>+</sup> cells has been shown in a number of in vitro tests (reviewed by Wang and Borysiewicz (217)). In bone marrow and HIV infection a soluble factor-mediated suppression by CD8<sup>+</sup>CD57<sup>+</sup> T cells has been reported (230, 236).

In this regard it is of particular interest that Prud'Homme and his group observed an association between long-term renal allograft function and an expansion of the CD8<sup>+</sup>CD57<sup>+</sup> cells. In vitro these cells suppressed mixed lymphocyte culture responses, pokeweed mitogen-induced IgG secretion and supernatants from cell cultures were also suppressive (213). A number of studies reported potential suppressor and regulatory functions of various cell subsets in organ transplantation (32, 34, 157, 214, 215, 222, 237, 238). However, despite the multitude of efforts no clear-cut regulatory cell and/or soluble factor could be identified.

Recently, the concept of regulatory T cells has experienced a renaissance due to their role in preventing auto-immunity. Multiple types of regulatory cells have been described. The best characterized are the CD4<sup>+</sup>CD25<sup>+</sup> lymphocytes (239, 240). In solid organ transplantation, Suciu-Foca and co-workers showed that CD8<sup>+</sup>CD28<sup>-</sup> alloantigen-specific T suppressor cells induced the up-regulation of inhibitory receptors on monocytes and dendritic cells, rendering these antigen-presenting cells tolerogenic (241). This distinct cell population was expanded in heart and liver transplant patients. It uses antigen-presenting cells as a bridge to suppress antigen-specific CD4<sup>+</sup> T cell responses. In a second paper they further classified this population and indicated that CD8<sup>+</sup>CD28<sup>-</sup>CD27<sup>+</sup> T cells are the subset with regulatory function in allograft recipients (242).

In respect to these phenotypic classifications it is of particular interest that the co-stimulatory molecule CD28

**Table 3.** Phenotypic changes associated with transplantation and aging

Characteristic change	Transplantation	Aging
Expansion of CD8 <sup>+</sup> CD57 <sup>+</sup> T cells	32-35, 147, 172, 199, 213-216	47, 56, 57, 252
Expansion of CD8 <sup>+</sup> CD28 <sup>-</sup> T cells	199, 223, 241, 242	47, 49, 56, 57, 253
Shift from naïve to memory T cells	173, 181, 183, 199, 261	47-49
Contracted repertoire	199, 262-264	47, 49, 233, 246
Telomere shortening	247-249	47, 56, 246, 251

shows a reciprocal expression with the marker CD57. CD28 and CD57 are mutually exclusively expressed on CD8<sup>+</sup> T cells. Only around 1% of CD8<sup>+</sup> T cells express both markers (57, 182, 223, 234).

This sheds new light on the expansion of specific cell subsets like CD8<sup>+</sup>CD57<sup>+</sup> and their role in organ transplantation. Comparable to CD57<sup>+</sup> T cells, CD28<sup>-</sup> cells are highly differentiated, clonally expanded T cells (182, 243). In a reciprocal manner to CD57 the molecule CD28 is down-regulated after Ag-stimulation (244, 245), the CD28<sup>-</sup> cells show shortened telomeres (246) and a diminished proliferative capacity (233).

Fagnoni analyzed the regeneration of lymphocyte subsets and expression in particular of CD28 after high-dose chemotherapy in women with breast cancer. As discussed above, her findings corroborate the kinetics and regeneration pathways of homeostasis-driven proliferation. In addition the data are particularly helpful, as the regeneration occurred without the potential impact of an allograft or concomitant immunosuppression and evidence of CMV infections (182). The described regeneration kinetics and expansion of the CD8<sup>+</sup>CD28<sup>-</sup> T cells fit very well with the dynamics of the CD8<sup>+</sup>CD57<sup>+</sup> T cell subset described above in the transplant patients. The CD8<sup>+</sup> T cells recovered within 3 months, reaching higher levels than before depletion. The expansion was mainly due to an increase in CD8<sup>+</sup>CD28<sup>-</sup> T cells, which may persist permanently. They concluded, that the expansion of CD28<sup>-</sup>CD57<sup>+</sup> cells can account for the post-treatment early increase of total CD8<sup>+</sup> T cells, as described also by Mackall (182, 198).

In general, transplantation and predominantly lymphopenia-induced proliferation are associated with the expansion of specific subpopulations of highly differentiated cells. These cells seem to have a regulatory function. Further phenotypic and functional studies are needed to precisely characterize the generation and role of these cells in transplantation.

## 6.3. Accelerated aging of the immune system

The phenotypic changes in the lymphocyte subsets of transplant patients have many similarities to those seen in elderly people (see table 3).

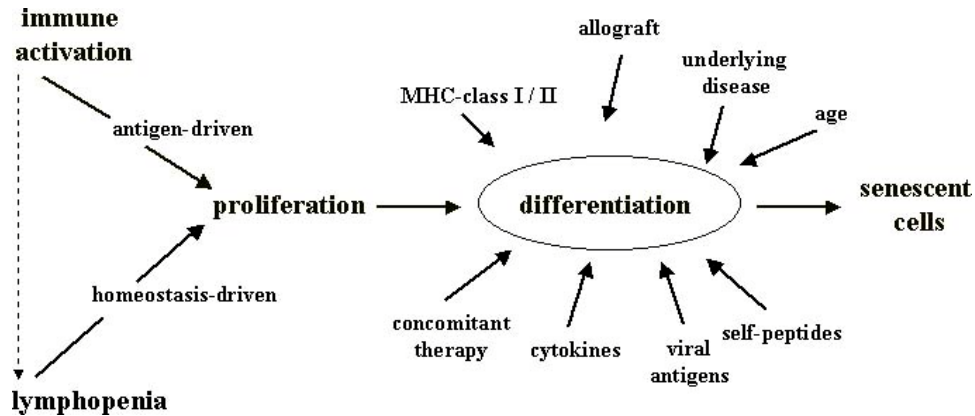
In transplant patients, T cells show a shift from naïve to memory phenotype. The subset of CD8<sup>+</sup> T cells is expanded and characterized by an up-regulation of the CD57 and a down-regulation of the CD28 surface molecules. The increased CD8<sup>+</sup> T cell population is

clonally expanded and has a restricted TCR repertoire. In addition, recent studies in recipients of allogeneic bone marrow transplants demonstrated an accelerated telomere shortening (247-249).

In elderly subjects, the frequency of naïve T cells is reduced. The proportion of memory T cells increases with aging (49). The co-stimulatory molecule CD28 and the glycoprotein CD57 are used as biomarkers of aging for human lymphocytes (47, 57). A significant correlation between age and decrease in CD28 and reciprocal increase in CD57 expression on CD8<sup>+</sup> T cells has been reported (57). Solana and his group showed that aging is associated with an increased expression of NK markers, such as CD57 on T cells (56). CD57<sup>+</sup> and/or CD28<sup>-</sup> CD8<sup>+</sup> T cells are highly differentiated cells. They have a low proliferative capacity and significantly shorter telomere lengths. Therefore these cells show the characteristics of replicative senescence, i.e. the inability to divide again in culture (47, 56, 246, 250, 251). A very recent study of HIV-specific CD8<sup>+</sup> T cells characterized CD57 surface expression as a marker of replicative senescence (252). The age-dependent accumulation of CD8<sup>+</sup>CD28<sup>-</sup> T cells might be due to their increased resistance to apoptosis (253). Restrictions in the TCR repertoire and oligoclonal expansions are prevalent in the CD28<sup>-</sup> and the CD57<sup>+</sup> T cell subset (47, 49, 233, 246).

These similarities in immunophenotypic changes between transplant patients and elderly people suggest that transplantation is associated with an accelerated aging of the immune system. Halloran introduced the concept of 'accelerated senescence' into transplant medicine (254). In this model, the cumulative burden of injury and age exhausts the ability of graft parenchymal cells to repair and maintain tissue integrity, eventually leading to chronic allograft nephropathy. In an analogous way, the term 'accelerated immunosenescence' may be used for the phenotypic findings described above and the potential underlying mechanisms causing an accelerated aging of the immune system.

The concept of premature aging of the immune system has been described before in two disease entities, human immunodeficiency virus (HIV) infection and rheumatoid arthritis (RA) (20, 255). Despite the marked differences between transplantation, HIV infection, and RA, all three disease entities are characterized by a chronic immune activation and inflammation. Figure 2 shows that immune activation induces T cell proliferation and differentiation (20, 207, 256, 257). The accumulation of cells showing the phenotype of terminal differentiation and the functional state of replicative senescence might be the end-product reflecting the cumulative burden of immune



**Figure 2.** Possible pathways and factors leading to an accelerated aging of the immune system in transplant patients.

activation. The proliferation and differentiation of the cells is influenced by the transplant-specific micro-environment and might explain the expansion of specific cell subsets such as  $CD8^+CD57^+CD28^-$  T cells. Chronic immune activation, both due to adaptive and innate/inflammatory stimuli, and the associated increased cell turnover are likely to be key elements in this process. Lymphocyte depletion is likely to increase the number of cells entering this proliferation and differentiation cycle.

Recent findings on the proliferation pathways support this model (see also section 3.1.). In antigen-driven proliferation, the individual cell seems to underlie an all-or-nothing response to activation, i.e. once activated it undergoes a programmed proliferation, contraction and memory differentiation. This 'auto-pilot' response might drive the single cell into replicative senescence (43, 44, 47). Homeostasis-driven proliferation seems to trigger a comparable programmed response just as antigen-driven proliferation leading to the same memory-phenotype cells (10, 31). Disease and treatment characteristics determine the relative extent and overlap of antigen- versus homeostasis-driven proliferation (46, 190).

The functional role and implications of these changes need further studies. Overall, transplant patients have a weaker response to vaccination and an increased susceptibility to infections and tumors. On the other hand, aging seems to be associated with a lower incidence in acute rejection episodes (258-260). Prospective and longitudinal studies investigating sequentially the long-term phenotypic and functional changes in lymphocyte populations in regard to different treatment strategies and age groups are needed. These efforts must also integrate clinical practice with basic research in order to analyze the data critically. Transplantation and the accelerated changes associated might offer an ideal window to learn more about both, aging and the immune system.

## 7. SUMMARY AND PERSPECTIVE

Transplantation and immunosuppression are associated with characteristic changes in the expression of surface molecules. The analysis of these perturbations has

to take into account both methodological limitations of the fluorescence-based approach and physiologic changes occurring during lymphocyte development and aging. Phenotypic changes are used to monitor and diagnose immune responses associated with transplantation, in particular rejection and infection. However, flow cytometric monitoring is not routinely used for diagnostic purposes due to its lack of specificity.

The immunosuppressive agents can be divided into two groups in regard to their effect on surface molecules. Small molecule drugs act primarily on intracellular pathways and only indirectly on surface markers. The mono- and polyclonal antibodies use surface molecules as direct targets. Monitoring of the changes in expression of surface markers is used to adjust and individualize the degree of immunosuppression.

The most pronounced effects on immune phenotypes are seen with lymphocyte depleting agents. The creation of an 'empty space' induces cell regeneration by homeostasis-driven proliferation. This regeneration is associated with characteristic changes in the phenotypes of the individual lymphocyte subpopulations. Thymic involution, i.e. age, is a major factor in determining the extent of changes seen for the regeneration of T cells, both in regard to the ratio of  $CD4^+$  to  $CD8^+$  and of naïve to memory cells. Following depletion, immune reconstitution leads to a novel homeostasis within the different lymphocyte subsets. This novel homeostasis is characterized by a shift towards more differentiated cell phenotypes and an expansion of specific cell subsets. These cell populations have long been associated with regulator and suppressor functions in transplantation. A recent renaissance in the concept of regulatory cells has lead to an intense search for cell populations with specific phenotypes inducing tolerance.

The overall changes in phenotypes seen in the different lymphocyte subsets of transplant patients show a striking similarity to those seen in elderly patients. In addition comparable phenotypic patterns are also seen with disease entities characterized by chronic immune activation. It is feasible that chronic immune activation in

transplantation, due to the increased antigenic and inflammatory load, leads to proliferation and an expansion of terminally differentiated T cells with low proliferative capacity. Therefore, an accelerated aging of the immune system is hypothesized on the basis of the phenotypic changes seen with immunosuppression in transplant patients.

Future studies will further characterize these changes. The advances in fluorescence-based technologies will improve the identification of 'fine' subsets with distinct phenotypic and functional characteristics. The knowledge about reconstitution kinetics, pathways, and induction of specific cell subsets will facilitate the individualization of immunosuppressive therapies. This might be one approach to achieve clinical tolerance.

## 8. ACKNOWLEDGEMENT

I would like to thank Walt Zybko and Alan Salama for their suggestions and corrections.

## 9. REFERENCES

- Reinherz E. L. & S. F. Schlossman: The differentiation and function of human T lymphocytes. *Cell* 19, 821-827 (1980)
- W. E. Paul: Fundamental Immunology. Lippincott-Raven, NY (1998)
- De Rosa S. C, J. M. Brenchley & M. Roederer: Beyond six colors: A new era in flow cytometry. *Nat Med* 9, 112-117 (2003)
- Shanahan T: Application of flow cytometry in transplantation medicine. *Immunol Invest* 26, 91-101 (1997)
- Zola H: Immunological applications of flow cytometry. *J Immunol Methods* 243, 1-2 (2000)
- Mason D, P. Andre, A. Bensussan, C. Buckley, C. Civin, E. Clark, M. de Haas, S. Goyert, M. Hadam, D. Hart, V. Horejsi, S. Meuer, J. Morrissey, R. Schwartz-Albiez, S. Shaw, D. Simmons, M. Uguccioni, E. van der Schoot, E. Vivier & H. Zola: CD antigens 2001. *J Leukoc Biol* 70, 685-690 (2001)
- Goldsby K, Osborne: Kuby Immunology. WH Freeman and Company, NY (2000)
- Reckzeh B, S. O. Grebe, I. Rhiele, A. Neubauer & T. F. Mueller: Monitoring of ATG therapy by flow cytometry: comparison of one single- platform and two different dual-platform methods. *Transplant Proc* 33, 2234-2236 (2001)
- De Rosa S. C, L. A. Herzenberg & M. Roederer: 11-color, 13-parameter flow cytometry: identification of human naive T cells by phenotype, function, and T-cell receptor diversity. *Nat Med* 7, 245-248 (2001)
- Murali-Krishna K & R. Ahmed: Cutting edge: naive T cells masquerading as memory cells. *J Immunol* 165, 1733-1737 (2000)
- Ahmed R & D. Gray: Immunological memory and protective immunity: understanding their relation. *Science* 272, 54-60 (1996)
- Sprent J & C. D. Surh: Generation and maintenance of memory T cells. *Curr Opin Immunol* 13, 248-254 (2001)
- Dai Z & F. G. Lakkis: Cutting edge: Secondary lymphoid organs are essential for maintaining the CD4, but not CD8, naive T cell pool. *J Immunol* 167, 6711-6715 (2001)
- Mosmann T. R, H. Cherwinski, M. W. Bond, M. A. Giedlin & R. L. Coffman: Two types of murine helper T cell clone. I. Definition according to profiles of lymphokine activities and secreted proteins. *J Immunol* 136, 2348-2357 (1986)
- Gray D: Thanks for the memory. *Nat Immunol* 1, 11-12 (2000)
- Veiga-Fernandes H, U. Walter, C. Bourgeois, A. McLean & B. Rocha: Response of naive and memory CD8+ T cells to antigen stimulation in vivo. *Nat Immunol* 1, 47-53 (2000)
- Garcia S, J. DiSanto & B. Stockinger: Following the development of a CD4 T cell response in vivo: from activation to memory formation. *Immunity* 11, 163-171 (1999)
- Opferman J. T, B. T. Ober & P. G. Ashton-Rickardt: Linear differentiation of cytotoxic effectors into memory T lymphocytes. *Science* 283, 1745-1748 (1999)
- Hamann D, M. T. Roos & R. A. van Lier: Faces and phases of human CD8 T-cell development. *Immunol Today* 20, 177-180 (1999)
- Appay V & S. L. Rowland-Jones: Premature ageing of the immune system: the cause of AIDS? *Trends Immunol* 23, 580-585 (2002)
- Cho B. K, C. Wang, S. Sugawa, H. N. Eisen & J. Chen: Functional differences between memory and naive CD8 T cells. *Proc Natl Acad Sci U S A* 96, 2976-2981 (1999)
- Oehen S & K. Brduscha-Riem: Differentiation of naive CTL to effector and memory CTL: correlation of effector function with phenotype and cell division. *J Immunol* 161, 5338-5346 (1998)
- Kimmig S, G. K. Przybylski, C. A. Schmidt, K. Laurisch, B. Mowes, A. Radbruch & A. Thiel: Two subsets of naive T helper cells with distinct T cell receptor excision circle content in human adult peripheral blood. *J Exp Med* 195, 789-794 (2002)
- Appay V, P. R. Dunbar, M. Callan, P. Klennerman, G. M. Gillespie, L. Papagno, G. S. Ogg, A. King, F. Lechner, C. A. Spina, S. Little, D. V. Havlir, D. D. Richman, N. Gruener, G. Pape, A. Waters, P. Easterbrook, M. Salio, V. Cerundolo, A. J. McMichael & S. L. Rowland-Jones: Memory CD8+ T cells vary in differentiation phenotype in different persistent virus infections. *Nat Med* 8, 379-385 (2002)
- Mitra D. K, S. C. De Rosa, A. Luke, A. Balamurugan, B. K. Khaitan, J. Tung, N. K. Mehra, A. I. Terr, A. O'Garra, L. A. Herzenberg & M. Roederer: Differential representations of memory T cell subsets are characteristic of polarized immunity in leprosy and atopic diseases. *Int Immunol* 11, 1801-1810 (1999)
- Murali-Krishna K, L. L. Lau, S. Sambhara, F. Lemonnier, J. Altman & R. Ahmed: Persistence of memory CD8 T cells in MHC class I-deficient mice. *Science* 286, 1377-1381 (1999)
- Tanchot C, F. A. Lemonnier, B. Perarnau, A. A. Freitas & B. Rocha: Differential requirements for survival and proliferation of CD8 naive or memory T cells. *Science* 276, 2057-2062 (1997)
- Kieper W. C & S. C. Jameson: Homeostatic expansion and phenotypic conversion of naive T cells in response to self peptide/MHC ligands. *Proc Natl Acad Sci U S A* 96, 13306-13311 (1999)

29. Ernst B, D. S. Lee, J. M. Chang, J. Sprent & C. D. Surh: The peptide ligands mediating positive selection in the thymus control T cell survival and homeostatic proliferation in the periphery. *Immunity* 11, 173-181 (1999)
30. Oehen S & K. Brduscha-Riem: Naive cytotoxic T lymphocytes spontaneously acquire effector function in lymphocytopenic recipients: A pitfall for T cell memory studies? *Eur J Immunol* 29, 608-614 (1999)
31. Ge Q, H. Hu, H. N. Eisen & J. Chen: Naive to memory T-cell differentiation during homeostasis-driven proliferation. *Microbes Infect* 4, 555-558 (2002)
32. Gorochoy G, P. Debre, V. Leblond, B. Sadat-Sowti, F. Sigaux & B. Autran: Oligoclonal expansion of CD8+ CD57+ T cells with restricted T-cell receptor beta chain variability after bone marrow transplantation. *Blood* 83, 587-595 (1994)
33. Mackall C. L, T. A. Fleisher, M. R. Brown, M. P. Andrich, C. C. Chen, I. M. Feuerstein, M. E. Horowitz, I. T. Magrath, A. T. Shad, S. M. Steinberg & et al.: Age, thymopoiesis, and CD4+ T-lymphocyte regeneration after intensive chemotherapy. *N Engl J Med* 332, 143-149 (1995)
34. Autran B, V. Leblond, B. Sadat-Sowti, E. Lefranc, P. Got, L. Sutton, J. L. Binet & P. Debre: A soluble factor released by CD8+CD57+ lymphocytes from bone marrow transplanted patients inhibits cell-mediated cytotoxicity. *Blood* 77, 2237-2241 (1991)
35. Fukuda H, H. Nakamura, N. Tominaga, H. Teshima, A. Hiraoka, H. Shibata & T. Masaoka: Marked increase of CD8+S6F1+ and CD8+CD57+ cells in patients with graft-versus-host disease after allogeneic bone marrow transplantation. *Bone Marrow Transplant* 13, 181-185 (1994)
36. Goldrath A. W, L. Y. Bogatzki & M. J. Bevan: Naive T cells transiently acquire a memory-like phenotype during homeostasis-driven proliferation. *J Exp Med* 192, 557-564 (2000)
37. Ge Q, H. Hu, H. N. Eisen & J. Chen: Different contributions of thymopoiesis and homeostasis-driven proliferation to the reconstitution of naive and memory T cell compartments. *Proc Natl Acad Sci U S A* 99, 2989-2994 (2002)
38. Wu C. Y, J. R. Kirman, M. J. Rotte, D. F. Davey, S. P. Perfetto, E. G. Rhee, B. L. Freidag, B. J. Hill, D. C. Douek & R. A. Seder: Distinct lineages of T(H)1 cells have differential capacities for memory cell generation in vivo. *Nat Immunol* 3, 852-858 (2002)
39. Dooks H & A. K. Abbas: Life and death in effector T cells. *Nat Immunol* 3, 797-798 (2002)
40. Lanzavecchia A & F. Sallusto: Dynamics of T lymphocyte responses: intermediates, effectors, and memory cells. *Science* 290, 92-97 (2000)
41. Kaech S. M & R. Ahmed: Memory CD8+ T cell differentiation: initial antigen encounter triggers a developmental program in naive cells. *Nat Immunol* 2, 415-422 (2001)
42. van Stipdonk M. J, E. E. Lemmens & S. P. Schoenberger: Naive CTLs require a single brief period of antigenic stimulation for clonal expansion and differentiation. *Nat Immunol* 2, 423-429 (2001)
43. Bevan M. J & P. J. Fink: The CD8 response on autopilot. *Nat Immunol* 2, 381-382 (2001)
44. Blattman J. N, L. E. Cheng & P. D. Greenberg: CD8(+) T cell responses: it's all downhill after their prime. *Nat Immunol* 3, 601-602 (2002)
45. Whitmire J. K & R. Ahmed: The economy of T-cell memory: CD4+ recession in times of CD8+ stability? *Nat Med* 7, 892-893 (2001)
46. Prlic M & S. C. Jameson: Homeostatic expansion versus antigen-driven proliferation: common ends by different means? *Microbes Infect* 4, 531-537 (2002)
47. Pawelec G, Y. Barnett, R. Forsey, D. Frasca, A. Globerson, J. McLeod, C. Caruso, C. Franceschi, T. Fulop, S. Gupta, E. Mariani, E. Mocchegiani & R. Solana: T cells and aging, January 2002 update. *Front Biosci* 7, d1056-1183 (2002)
48. M. E. S. Weksler & R. Ai-hao: Aging and the immune system. In: Clinical Immunology. Principles and Practice, vol 1. Eds: Rich R. R. F, Schwartz T.A, Shearer B. D, Strober W.T, Mosby Year Book Inc., NY (1996)
49. Globerson A & R. B. Effros: Ageing of lymphocytes and lymphocytes in the aged. *Immunol Today* 21, 515-521 (2000)
50. Linton P. J, L. Haynes, L. Tsui, X. Zhang & S. Swain: From naive to effector--alterations with aging. *Immunol Rev* 160, 9-18 (1997)
51. Utsuyama M, K. Hirokawa, C. Kurashima, M. Fukayama, T. Inamatsu, K. Suzuki, W. Hashimoto & K. Sato: Differential age-change in the numbers of CD4+CD45RA+ and CD4+CD29+ T cell subsets in human peripheral blood. *Mech Ageing Dev* 63, 57-68 (1992)
52. Franceschi C, M. Bonafe & S. Valensin: Human immunosenescence: the prevailing of innate immunity, the failing of clonotypic immunity, and the filling of immunological space. *Vaccine* 18, 1717-1720 (2000)
53. Douek D. C, R. D. McFarland, P. H. Keiser, E. A. Gage, J. M. Massey, B. F. Haynes, M. A. Polis, A. T. Haase, M. B. Feinberg, J. L. Sullivan, B. D. Jamieson, J. A. Zack, L. J. Picker & R. A. Koup: Changes in thymic function with age and during the treatment of HIV infection. *Nature* 396, 690-695 (1998)
54. Mackall C. L, C. V. Bare, L. A. Granger, S. O. Sharrow, J. A. Titus & R. E. Gress: Thymic-independent T cell regeneration occurs via antigen-driven expansion of peripheral T cells resulting in a repertoire that is limited in diversity and prone to skewing. *J Immunol* 156, 4609-4616 (1996)
55. Mackall C. L & R. E. Gress: Thymic aging and T-cell regeneration. *Immunol Rev* 160, 91-102 (1997)
56. Tarazona R, O. DelaRosa, C. Alonso, B. Ostos, J. Espejo, J. Pena & R. Solana: Increased expression of NK cell markers on T lymphocytes in aging and chronic activation of the immune system reflects the accumulation of effector/senescent T cells. *Mech Ageing Dev* 121, 77-88 (2000)
57. Merino J, M. A. Martinez-Gonzalez, M. Rubio, S. Inoges, A. Sanchez-Ibarrola & M. L. Subira: Progressive decrease of CD8high+ CD28+ CD57- cells with ageing. *Clin Exp Immunol* 112, 48-51 (1998)
58. van Rood Y, E. Goulmy, E. Blokland, J. Pool, J. van Rood & H. van Houwelingen: Month-related variability in immunological test results; implications for immunological follow-up studies. *Clin Exp Immunol* 86, 349-354 (1991)
59. Laurence J: T-cell subsets in health, infectious disease, and idiopathic CD4+ T lymphocytopenia. *Ann Intern Med* 119, 55-62 (1993)

60. Malone J. L., T. E. Simms, G. C. Gray, K. F. Wagner, J. R. Burge & D. S. Burke: Sources of variability in repeated T-helper lymphocyte counts from human immunodeficiency virus type 1-infected patients: total lymphocyte count fluctuations and diurnal cycle are important. *J Acquir Immune Defic Syndr* 3, 144-151 (1990)
61. Levi F, C. Canon, J. P. Blum, A. Reinberg & G. Mathe: Large-amplitude circadian rhythm in helper:suppressor ratio of peripheral blood lymphocytes. *Lancet* 2, 462-463 (1983)
62. Giorgi J. V & R. Detels: T-cell subset alterations in HIV-infected homosexual men: NIAID Multicenter AIDS cohort study. *Clin Immunol Immunopathol* 52, 10-18 (1989)
63. Westermann J & R. Pabst: Lymphocyte subsets in the blood: a diagnostic window on the lymphoid system? *Immunol Today* 11, 406-410 (1990)
64. Springer T. A: Traffic signals for lymphocyte recirculation and leukocyte emigration: the multistep paradigm. *Cell* 76, 301-314 (1994)
65. McLean A. R & C. A. Michie: In vivo estimates of division and death rates of human T lymphocytes. *Proc Natl Acad Sci U S A* 92, 3707-3711 (1995)
66. Asquith B, C. Debaq, D. C. Macallan, L. Willems & C. R. Bangham: Lymphocyte kinetics: the interpretation of labelling data. *Trends Immunol* 23, 596-601 (2002)
67. Roederer M: Getting to the HAART of T cell dynamics. *Nat Med* 4, 145-146 (1998)
68. Cosimi A. B, R. B. Colvin, R. C. Burton, R. H. Rubin, G. Goldstein, P. C. Kung, W. P. Hansen, F. L. Delmonico & P. S. Russell: Use of monoclonal antibodies to T-cell subsets for immunologic monitoring and treatment in recipients of renal allografts. *N Engl J Med* 305, 308-314 (1981)
69. Schooley R. T, M. S. Hirsch, R. B. Colvin, A. B. Cosimi, N. E. Tolloff-Rubin, R. T. McCluskey, R. C. Burton, P. S. Russell, J. T. Herrin, F. L. Delmonico, J. V. Giorgi, W. Henle & R. H. Rubin: Association of herpesvirus infections with T-lymphocyte-subset alterations, glomerulopathy, and opportunistic infections after renal transplantation. *N Engl J Med* 308, 307-313 (1983)
70. Valeri M, A. Piazza, N. Torlone, E. Poggi, D. Adorno & C. U. Casciani: Immunologic monitoring in kidney transplantation. *Transplant Proc* 23, 2275-2276 (1991)
71. Pham B. N, J. F. Mosnier, F. Walker, C. Njapoum, F. Bougy, C. Degott, S. Erlinger, J. H. Cohen & F. Degos: Flow cytometry CD4+/CD8+ ratio of liver-derived lymphocytes correlates with viral replication in chronic hepatitis B. *Clin Exp Immunol* 97, 403-410 (1994)
72. Siegel D. L, I. Fox, D. C. Dafoe, M. Power, M. Asplund, L. Zellers, C. F. Barker & M. B. Prystowsky: Discriminating rejection from CMV infection in renal allograft recipients using flow cytometry. *Clin Immunol Immunopathol* 51, 157-171 (1989)
73. Henny F. C, A. van Es, P. J. Oljans, W. M. Baldwin, H. J. Tanke, L. A. van Es & L. C. Paul: Prognostic value of T lymphocyte subset ratios for renal transplant survival in patients on different immunosuppressive regimens. *Clin Exp Immunol* 65, 373-380 (1986)
74. Kiparski A, D. Frei, W. Fierz, G. Frei, G. Uhlschmid, F. Largiader & U. Binswanger: [The effect of rejection crises and immunosuppressive therapy on the lymphocyte subpopulations of patients after kidney transplantation]. *Klin Wochenschr* 68, 408-414 (1990)
75. Olausson M, L. Mjornstedt, L. Lindholm & H. Brynner: Suppressor cells in antithymocyte globulin-induced transplantation tolerance in the adult rat. *Int Arch Allergy Appl Immunol* 75, 184-187 (1984)
76. Olausson M, L. Wramner, B. Kjellson, L. Mjornstedt & T. Soderstrom: Alteration in T cell subset phenotypes as cell markers for rejection or long-term allograft acceptance in allografted rats treated with antithymocyte globulin. *Int Arch Allergy Immunol* 101, 431-436 (1993)
77. Olausson M, L. Mjornstedt, I. Blohme, L. Wramner & T. Soderstrom: Is a persistently low level of T-helper cells a marker of rejection in renal transplant patients? *Transplant Proc* 24, 300-302 (1992)
78. Trindade H, A. G. da Costa, H. Carvalho, A. Lima, A. Correia, M. J. Sampaio, J. A. Caetano & J. R. Pena: Lymphocyte populations in peripheral blood after kidney transplantation. *Transplant Proc* 24, 2553-2555 (1992)
79. Hoshinaga K, T. Mohanakumar, N. L. Wood, S. Szentpetery & R. R. Lower: Increase in transferrin receptors as a marker for rejection and infection in human cardiac transplantation. *Transplant Proc* 19, 2534-2536 (1987)
80. Garcia-Alonso A. M, A. Minguela, M. Muro, J. Ontanon, A. Torio, L. Marin, P. Lopez-Segura, M. Miras & M. R. Alvarez-Lopez: CD28 expression on peripheral blood T lymphocytes after orthotopic liver transplant: upregulation in acute rejection. *Hum Immunol* 53, 64-72 (1997)
81. T. F. Mueller: Immunological monitoring following organ transplantation, Pabst Science Publishers, Lengerich (1999)
82. Haveman J. W, A. P. van den Berg, J. M. van den Berk, G. Mesander, M. J. Slooff, L. H. de Leij & T. H. The: Low HLA-DR expression on peripheral blood monocytes predicts bacterial sepsis after liver transplantation: relation with prednisolone intake. *Transpl Infect Dis* 1, 146-152 (1999)
83. Reinke P & H. D. Volk: Diagnostic and predictive value of an immune monitoring program for complications after kidney transplantation. *Urol Int* 49, 69-75 (1992)
84. Deenitchina S. S, T. Ando, S. Okuda, N. Kinukawa, H. Hirakata, A. Nagashima & M. Fujishima: Cellular immunity in hemodialysis patients: a quantitative analysis of immune cell subsets by flow cytometry. *Am J Nephrol* 15, 57-65 (1995)
85. Matsumoto Y, T. Shinzato, I. Takai, A. Nishimoto, S. Nakai, T. Sakai, I. Amano & K. Maeda: Peripheral deletion of gamma/delta T cells in haemodialysis patients. *Nephrol Dial Transplant* 13, 2861-2866 (1998)
86. O'Regan A. W, J. Sadeh & J. S. Berman: The effect of unfractionated heparin on circulating lymphocyte counts and subsets in humans. *Thromb Res* 106, 31-33 (2002)
87. Dambrin C, J. Klupp & R. E. Morris: Pharmacodynamics of immunosuppressive drugs. *Curr Opin Immunol* 12, 557-562 (2000)
88. Stalder M, T. Birsan, B. Holm, M. Haririfar, J. Scandling & R. E. Morris: Quantification of immunosuppression by flow cytometry in stable renal transplant recipients. *Ther Drug Monit* 25, 22-27 (2003)



89. Sindhi R, J. Allaert, D. Gladding, P. Haaland, B. Koppelman, J. Dunne & S. Sehgal: Modeling individual variation in biomarker response to combination immunosuppression with stimulated lymphocyte responses-potential clinical implications. *J Immunol Methods* 272, 257-272 (2003)
90. Sindhi R, S. Webber, R. Goyal, J. Reyes, R. Venkataramanan & L. Shaw: Pharmacodynamics of sirolimus in transplanted children receiving tacrolimus. *Transplant Proc* 34, 1960 (2002)
91. Sindhi R, M. F. LaVia, E. Paulling, J. McMichael, G. Burckart, S. Shaw, L. A. Sindhi, R. Livingston, S. Sehgal & J. Jaffe: Stimulated response of peripheral lymphocytes may distinguish cyclosporine effect in renal transplant recipients receiving a cyclosporine+rapamycin regimen. *Transplantation* 69, 432-436 (2000)
92. Denton M. D, C. C. Magee & M. H. Sayegh: Immunosuppressive strategies in transplantation. *Lancet* 353, 1083-1091 (1999)
93. Carpenter C. B: Immunosuppression in organ transplantation. *N Engl J Med* 322, 1224-1226 (1990)
94. Gourishankar S, P. Turner & P. Halloran: New developments in immunosuppressive therapy in renal transplantation. *Expert Opin Biol Ther* 2, 483-501 (2002)
95. Halloran P: Mechanisms of current immunosuppression. *Transplantation Review and Update* (2001)
96. Jenkins M. K, R. H. Schwartz & D. M. Pardoll: Effects of cyclosporine A on T cell development and clonal deletion. *Science* 241, 1655-1658 (1988)
97. Gao E. K, D. Lo, R. Cheney, O. Kanagawa & J. Sprent: Abnormal differentiation of thymocytes in mice treated with cyclosporin A. *Nature* 336, 176-179 (1988)
98. Kosugi A, S. O. Sharrow & G. M. Shearer: Effect of cyclosporin A on lymphopoiesis. I. Absence of mature T cells in thymus and periphery of bone marrow transplanted mice treated with cyclosporin A. *J Immunol* 142, 3026-3032 (1989)
99. Kosugi A, J. C. Zuniga-Pflucker, S. O. Sharrow, A. M. Kruisbeek & G. M. Shearer: Effect of cyclosporin A on lymphopoiesis. II. Developmental defects of immature and mature thymocytes in fetal thymus organ cultures treated with cyclosporin A. *J Immunol* 143, 3134-3140 (1989)
100. Kosugi A & G. M. Shearer: Effect of cyclosporin A on lymphopoiesis. III. Augmentation of the generation of natural killer cells in bone marrow transplanted mice treated with cyclosporin A. *J Immunol* 146, 1416-1421 (1991)
101. Hollander G. A, D. A. Fruman, B. E. Bierer & S. J. Burakoff: Disruption of T cell development and repertoire selection by calcineurin inhibition in vivo. *Transplantation* 58, 1037-1043 (1994)
102. Koprak S, A. Sirotina, H. Ok & F. J. Dumont: Depletion of the mature CD4+8- thymocyte subset by FK506 analogs correlates with their immunosuppressive and calcineurin inhibitory activities. *Transplantation* 61, 926-932 (1996)
103. Sumpio B. E, J. M. Dwyer & M. W. Flye: T-lymphocyte subsets in renal allograft recipients treated with cyclosporine and azathioprine. *Ann Surg* 205, 49-53 (1987)
104. Takahashi N, H. Nishida & J. Hoshi: Severe B cell depletion in newborns from renal transplant mothers taking immunosuppressive agents. *Transplantation* 57, 1617-1621 (1994)
105. Pilarski L. M, B. R. Yacyszyn & A. I. Lazarovits: Analysis of peripheral blood lymphocyte populations and immune function from children exposed to cyclosporine or to azathioprine in utero. *Transplantation* 57, 133-144 (1994)
106. Alamartine E, O. Sabido & F. C. Berthoux: The influence of prophylactic immunosuppressive regimens on natural killer and lymphokine-activated killer cells in renal transplant recipients. *Transplantation* 50, 969-973 (1990)
107. Melms A, G. Malcherek, U. Gern, N. Sommer, R. Weissert, H. Wietholter & H. J. Buhning: Thymectomy and azathioprine have no effect on the phenotype of CD4 T lymphocyte subsets in myasthenia gravis. *J Neurol Neurosurg Psychiatry* 56, 46-51 (1993)
108. Gummert J. F, M. J. Barten, S. W. Sherwood, T. van Gelder & R. E. Morris: Pharmacodynamics of immunosuppression by mycophenolic acid: inhibition of both lymphocyte proliferation and activation correlates with pharmacokinetics. *J Pharmacol Exp Ther* 291, 1100-1112 (1999)
109. Kimball J. A, M. D. Pescovitz, B. K. Book & D. J. Norman: Reduced human IgG anti-ATGAM antibody formation in renal transplant recipients receiving mycophenolate mofetil. *Transplantation* 60, 1379-1383 (1995)
110. Izeradjene K & J. P. Revillard: Apoptosis of superantigen-activated T cells induced by mycophenolate mofetil treatment. *Transplantation* 71, 118-125 (2001)
111. Godfrey D. I, J. F. Purton, R. L. Boyd & T. J. Cole: Glucocorticoids and the thymus: the view from the middle of the road. *Trends Immunol* 22, 243 (2001)
112. Godfrey D. I, J. F. Purton, R. L. Boyd & T. J. Cole: Stress-free T-cell development: glucocorticoids are not obligatory. *Immunol Today* 21, 606-611 (2000)
113. K. J. Wood: The Handbook of Transplant Immunology. MedSci Publications, Oxford (1995)
114. Raefsky E. L, P. Gascon, A. Gratwohl, B. Speck & N. S. Young: Biological and immunological characterization of ATG and ALG. *Blood* 68, 712-719 (1986)
115. Bonnefoy-Berard N, S. Fournel, L. Genestier, M. Flacher, L. Quemeneur & J. P. Revillard: In vitro functional properties of antithymocyte globulins: clues for new therapeutic applications? *Transplant Proc* 30, 4015-4017 (1998)
116. Bonnefoy-Berard N, C. Vincent & J. P. Revillard: Antibodies against functional leukocyte surface molecules in polyclonal antilymphocyte and antithymocyte globulins. *Transplantation* 51, 669-673 (1991)
117. Rebellato L. M., U. Gross, K. M. Verbanac & J. M. Thomas: A comprehensive definition of the major antibody specificities in polyclonal rabbit antithymocyte globulin. *Transplantation* 57, 685-694 (1994)
118. Bourdage J. S & D. M. Hamlin: Comparative polyclonal antithymocyte globulin and antilymphocyte/antilymphoblast globulin anti-CD antigen analysis by flow cytometry. *Transplantation* 59, 1194-1200 (1995)
119. Pistillo M. P, P. L. Tazzari, F. Bonifazi, G. Bandini, T. Kato, T. Matsui, K. Nishioka, R. Conte & G. B. Ferrara: Detection of a novel specificity (CTLA-4) in ATG/TMG

- globulins and sera from ATG-treated leukemic patients. *Transplantation* 73, 1295-1302 (2002)
120. Starzl T. E: Heterologous antilymphocyte globulin. *N Engl J Med* 279, 700-703 (1968)
121. Revillard J. P: Immunopharmacology of Thymoglobulin. *Graft* 2, S6-S9 (1999)
122. Preville X, L. Nicolas, M. Flacher & J. Revillard: A quantitative flow cytometry assay for the preclinical testing and pharmacological monitoring of rabbit antilymphocyte globulins (rATG). *J Immunol Methods* 245, 45-54 (2000)
123. Chatenoud L, M. F. Baudrihay, H. Kreis, G. Goldstein, J. Schindler & J. F. Bach: Human in vivo antigenic modulation induced by the anti-T cell OKT3 monoclonal antibody. *Eur J Immunol* 12, 979-982 (1982)
124. Bonnefoy-Berard N & J. P. Revillard: Mechanisms of immunosuppression induced by antithymocyte globulins and OKT3. *J Heart Lung Transplant* 15, 435-442 (1996)
125. Henell K. R & D. J. Norman: Monitoring OKT3 treatment: pharmacodynamic and pharmacokinetic measures. *Transplant Proc* 25, 83-85 (1993)
126. Henell K. R, A. Bakke, T. A. Kenny, J. A. Kimball, J. M. Barry & D. J. Norman: Degree of modulation of cell-surface CD3 by anti-lymphocyte therapies. *Transplant Proc* 23, 1070-1071 (1991)
127. Preville X, M. Flacher, B. LeMauff, S. Beauchard, P. Davelu, J. Tiollier & J. P. Revillard: Mechanisms involved in antithymocyte globulin immunosuppressive activity in a nonhuman primate model. *Transplantation* 71, 460-468 (2001)
128. Merion R. M, T. Howell & J. S. Bromberg: Partial T-cell activation and anergy induction by polyclonal antithymocyte globulin. *Transplantation* 65, 1481-1489 (1998)
129. Darby C. R, A. Bushell, P. J. Morris & K. J. Wood: Non-depleting anti-CD4 antibodies in transplantation. Evidence that modulation is far less effective than prolonged CD4 blockade. *Transplantation* 57, 1419-1426 (1994)
130. Kahan B. D, P. R. Rajagopalan & M. Hall: Reduction of the occurrence of acute cellular rejection among renal allograft recipients treated with basiliximab, a chimeric anti-interleukin-2-receptor monoclonal antibody. United States Simulect Renal Study Group. *Transplantation* 67, 276-284 (1999)
131. Kovarik J. M, G. Offner, M. Broyer, P. Niaudet, C. Loirat, M. Mentser, J. Lemire, J. F. Crocker, P. Cochat, G. Clark, C. Gerbeau, L. Chodoff, A. Korn & M. Hall: A rational dosing algorithm for basiliximab (Simulect) in pediatric renal transplantation based on pharmacokinetic-dynamic evaluations. *Transplantation* 74, 966-971 (2002)
132. Vincenti F, R. Kirkman, S. Light, G. Bumgardner, M. Pescovitz, P. Halloran, J. Neylan, A. Wilkinson, H. Ekberg, R. Gaston, L. Backman & J. Burdick: Interleukin-2-receptor blockade with daclizumab to prevent acute rejection in renal transplantation. Daclizumab Triple Therapy Study Group. *N Engl J Med* 338, 161-165 (1998)
133. Amlot P. L, E. Rawlings, O. N. Fernando, P. J. Griffin, G. Heinrich, M. H. Schreier, J. P. Castaigne, R. Moore & P. Sweny: Prolonged action of a chimeric interleukin-2 receptor (CD25) monoclonal antibody used in cadaveric renal transplantation. *Transplantation* 60, 748-756 (1995)
134. Junghans R. P, T. A. Waldmann, N. F. Landolfi, N. M. Avdalovic, W. P. Schneider & C. Queen: Anti-Tac-H, a humanized antibody to the interleukin 2 receptor with new features for immunotherapy in malignant and immune disorders. *Cancer Res* 50, 1495-1502 (1990)
135. Henell K. R & D. J. Norman: In vivo pharmacodynamics of Atgam induction immunosuppression in renal transplantation. *Transplant Proc* 27, 1052-1053 (1995)
136. Wing M. G, T. Moreau, J. Greenwood, R. M. Smith, G. Hale, J. Isaacs, H. Waldmann, P. J. Lachmann & A. Compston: Mechanism of first-dose cytokine-release syndrome by CAMPATH 1-H: involvement of CD16 (FcgammaRIII) and CD11a/CD18 (LFA-1) on NK cells. *J Clin Invest* 98, 2819-2826 (1996)
137. Hale G: The CD52 antigen and development of the CAMPATH antibodies. *Cytotherapy* 3, 137-143 (2001)
138. Elsner J, R. Hochstetter, K. Spiekermann & A. Kapp: Surface and mRNA expression of the CD52 antigen by human eosinophils but not by neutrophils. *Blood* 88, 4684-4693 (1996)
139. Genestier L, N. Bonnefoy-Berard & J. P. Revillard: Apoptosis of activated peripheral T cells. *Transplant Proc* 31, 33S-38S (1999)
140. Buysmann S, F. J. Bemelman, P. T. Schellekens, Y. van Kooyk, C. G. Figdor & I. J. ten Berge: Activation and increased expression of adhesion molecules on peripheral blood lymphocytes is a mechanism for the immediate lymphocytopenia after administration of OKT3. *Blood* 87, 404-411 (1996)
141. Genestier L, S. Fournel, M. Flacher, O. Assossou, J. P. Revillard & N. Bonnefoy-Berard: Induction of Fas (Apo-1, CD95)-mediated apoptosis of activated lymphocytes by polyclonal antithymocyte globulins. *Blood* 91, 2360-2368 (1998)
142. Monaco A. P, W. M. Abbott, H. B. Othersen, R. L. Simmons, M. L. Wood, M. H. Flax & P. S. Russell: Antiserum to lymphocytes: prolonged survival of canine renal allografts. *Science* 153, 1264-1267 (1966)
143. Woodruff M: Effect of antilymphocyte serum on suspensions of lymphocytes in vitro. *Nature* 168, 35-36 (1951)
144. Woodruff M: Effect of lymphocyte depletion by thoracic duct fistula and administration of anti-lymphocyte serum on the survival of skin homografts in rats. *Nature* 200, 702 (1963)
145. Mestre M, C. Gonzalez, J. M. Grino, A. Valls, J. Bonete, E. Mane, M. Corominas, J. Bas, A. Romeu & E. Buendia: Sequential monitoring of immunoregulatory T cell subsets in renal transplantation. *Transplant Proc* 24, 73-75 (1992)
146. Zaltzman J. S, E. H. Cole & J. Freedman: The effect of rabbit antithymocyte serum (RATS) and OKT3 on peripheral blood mononuclear cell subsets following renal transplantation. *Clin Transplant* 8, 516-522 (1994)
147. Mueller T. F, S. O. Grebe, M. C. Neumann, J. Heymanns, K. Radsak, H. Sprenger & H. Lange: Persistent long-term changes in lymphocyte subsets induced by polyclonal antibodies. *Transplantation* 64, 1432-1437 (1997)
148. Mueller T. F, S. O. Grebe, B. Reckzeh, A. Borutta, K. Radsak & H. Lange: Short- and long-term effects of polyclonal antibodies. *Transplant Proc* 31, 12S-15S (1999)
149. Ekberg H, N. H. Persson, M. Olsson, A. Lundell & A. Bredberg: Flow cytometric measurements in 104 anti-T-

- lymphocyte globulin treatments. *Transplant Proc* 24, 2551-2552 (1992)
150. Troppmann C, P. S. Almond, C. Fasola, E. Benedetti, R. W. Gruessner, D. L. Dunn, P. F. Gores, W. D. Payne, A. J. Matas & J. S. Najarian: Effect of Minnesota antilymphocyte globulin on T-lymphocytes and their subsets during induction therapy in pediatric renal transplantation. *Transplant Proc* 26, 26-27 (1994)
151. Brophy P. D, S. E. Thomas, K. D. McBryde & T. E. Bunchman: Comparison of polyclonal induction agents in pediatric renal transplantation. *Pediatr Transplant* 5, 174-178 (2001)
152. Zimmerman B, S. L. Swain & R. W. Dutton: Immunosuppressive ATS. V. Analysis of the effect of anti-thymocyte serum on T lymphocyte subsets. *J Immunol* 129, 515-520 (1982)
153. Bell L, C. Girardin, A. Sharma, P. Goodyer & B. Mazer: Lymphocyte subsets during and after rabbit anti-thymocyte globulin induction in pediatric renal transplantation: sustained T cell depletion. *Transplant Proc* 29, 6S-9S (1997)
154. Bonnefoy-Berard N, M. Flacher & J. P. Revillard: Antiproliferative effect of antilymphocyte globulins on B cells and B- cell lines. *Blood* 79, 2164-2170 (1992)
155. Bonnefoy-Berard N, L. Genestier, M. Flacher, J. P. Rouault, G. Lizard, M. Mutin & J. P. Revillard: Apoptosis induced by polyclonal antilymphocyte globulins in human B- cell lines. *Blood* 83, 1051-1059 (1994)
156. Wramner L, D. S. Robbins, B. Kjellsson, L. Mjornstedt, M. Olausson, H. Brynner & T. Soderstrom: Blood lymphocyte subsets in ATG-treated and allografted rats. *Transpl Int* 3, 55-58 (1990)
157. Maki T, M. Simpson & A. P. Monaco: Development of suppressor T cells by antilymphocyte serum treatment in mice. *Transplantation* 34, 376-381 (1982)
158. Knechtel S. J: Treatment with immunotoxin. *Philos Trans R Soc Lond B Biol Sci* 356, 681-689 (2001)
159. Calne R, S. D. Moffatt, P. J. Friend, N. V. Jamieson, J. A. Bradley, G. Hale, J. Firth, J. Bradley, K. G. Smith & H. Waldmann: Campath 1H allows low-dose cyclosporine monotherapy in 31 cadaveric renal allograft recipients. *Transplantation* 68, 1613-1616 (1999)
160. Waldmann H, G. Hale & S. Cobbald: Appropriate targets for monoclonal antibodies in the induction of transplantation tolerance. *Philos Trans R Soc Lond B Biol Sci* 356, 659-663 (2001)
161. Clark K. R, J. L. Forsythe, B. K. Shenton, T. W. Lennard, G. Proud & R. M. Taylor: Administration of ATG according to the absolute T lymphocyte count during therapy for steroid-resistant rejection. *Transpl Int* 6, 18-21 (1993)
162. Shenton B. K, M. D. White, A. E. Bell, K. Clark, K. M. Rigg, J. L. Forsythe, G. Proud & R. M. Taylor: The paradox of ATG monitoring in renal transplantation. *Transplant Proc* 26, 3177-3180 (1994)
163. Abouna G. M, I. H. al-Abdullah, D. Kelly-Sullivan, M. S. Kumar, J. Loose, K. Phillips, S. Yost & D. Seirka: Randomized clinical trial of antithymocyte globulin induction in renal transplantation comparing a fixed daily dose with dose adjustment according to T cell monitoring. *Transplantation* 59, 1564-1568 (1995)
164. Brun P, G. Sterkers, A. Maisin, K. Benali, V. Baudouin, M. Macher & C. Loirat: Flow-cytometry monitoring of antilymphocyte globulin therapy in pediatric renal transplantation. *Transplant Proc* 27, 1744-1745 (1995)
165. Gorrie M, G. Thomson, D. M. Lewis, M. Boyce, H. N. Riad, M. Beaman & A. J. Nicholls: Dose titration during anti-thymocyte globulin therapy: monitoring by CD3 count or total lymphocyte count? *Clin Lab Haematol* 19, 53-56 (1997)
166. Buchler M, G. Thibault, A. al Najjar, J. F. Valentin, A. Guerraoui, H. Nivet, P. Bardos & Y. Lebranchu: Monitoring of ATG therapy by flow cytometry and lymphocyte counts in renal transplantation. *Transplant Proc* 28, 2817-2818 (1996)
167. Djamali A, C. Turc-Baron, P. Portales, G. Leveson, G. Chong, J. Clot & G. Mourad: Low dose antithymocyte globulins in renal transplantation: daily versus intermittent administration based on T-cell monitoring. *Transplantation* 69, 799-805 (2000)
168. Peddi V. R, M. Bryant, P. Roy-Chaudhury, E. S. Woodle & M. R. First: Safety, efficacy, and cost analysis of thymoglobulin induction therapy with intermittent dosing based on CD3+ lymphocyte counts in kidney and kidney-pancreas transplant recipients. *Transplantation* 73, 1514-1518 (2002)
169. Forsythe J. L: ATG dosing - daily or less frequently? *Graft* 2, S10-S14 (1999)
170. Thomas F, H. M. Lee, J. S. Wolf, G. Mendez-Picon & J. Thomas: Monitoring and modulation of immune reactivity in human transplant recipients. *Surgery* 79, 408-413 (1976)
171. Thomas F, G. Mendez-Picon, J. Thomas & H. M. Lee: Individualized monitoring and modulation of immune reactivity using standardized antithymocyte globulin (ATG). *Transplant Proc* 11, 1429-1430 (1979)
172. Mueller T. F: Long-term T cell dynamics following the use of polyclonal and monoclonal antibodies. *Graft* 2, S15-S20 (1999)
173. Bas J, M. Mestre, J. M. Grinyo, E. Massip, J. Alsina, A. M. Castela, M. Corominas & E. Buendia: Peripheral blood lymphoid subsets and long-term clinical course of kidney recipients: a longitudinal study. *Cytometry* 34, 103-112 (1998)
174. Novitzky N, G. M. Davison, G. Hale & H. Waldmann: Immune reconstitution at 6 months following T-cell depleted hematopoietic stem cell transplantation is predictive for treatment outcome. *Transplantation* 74, 1551-1559 (2002)
175. Brett S, G. Baxter, H. Cooper, J. M. Johnston, J. Tite & N. Rapson: Repopulation of blood lymphocyte subpopulations in rheumatoid arthritis patients treated with the depleting humanized monoclonal antibody, CAMPATH-1H. *Immunology* 88, 13-19 (1996)
176. Reckzeh B, H. Merte, K. H. Pfluger, R. Pfab, M. Wolf & K. Havemann: Severe lymphocytopenia and interstitial pneumonia in patients treated with paclitaxel and simultaneous radiotherapy for non-small-cell lung cancer. *J Clin Oncol* 14, 1071-1076 (1996)
177. Freitas A & J. Chen: Introduction: regulation of lymphocyte homeostasis. *Microbes Infect* 4, 529-530 (2002)
178. I. Hanski: Metapopulation Ecology. Oxford University Press, Oxford (1999)

179. Goldrath A. W & M. J. Bevan: Low-affinity ligands for the TCR drive proliferation of mature CD8<sup>+</sup> T cells in lymphopenic hosts. *Immunity* 11, 183-190 (1999)
180. Tanchot C, A. Le Campion, S. Leament, N. Dautigny & B. Lucas: Naive CD4(+) lymphocytes convert to anergic or memory-like cells in T cell-deprived recipients. *Eur J Immunol* 31, 2256-2265 (2001)
181. Heitger A, H. Greinix, C. Mannhalter, D. Mayerl, H. Kern, J. Eder, F. M. Fink, D. Niederwieser & E. R. Panzer-Grumayer: Requirement of residual thymus to restore normal T-cell subsets after human allogeneic bone marrow transplantation. *Transplantation* 69, 2366-2373 (2000)
182. Fagnoni F. F, L. Lozza, C. Zibera, A. Zambelli, L. Ponchio, N. Gibelli, B. Oliviero, L. Pavesi, R. Gennari, R. Vescovini, P. Sansoni, G. Da Prada & G. Robustelli Della Cuna: T-cell dynamics after high-dose chemotherapy in adults: elucidation of the elusive CD8<sup>+</sup> subset reveals multiple homeostatic T-cell compartments with distinct implications for immune competence. *Immunology* 106, 27-37 (2002)
183. Heitger A, N. Neu, H. Kern, E. R. Panzer-Grumayer, H. Greinix, D. Nachbaur, D. Niederwieser & F. M. Fink: Essential role of the thymus to reconstitute naive (CD45RA<sup>+</sup>) T-helper cells after human allogeneic bone marrow transplantation. *Blood* 90, 850-857 (1997)
184. Davison G. M, N. Novitzky, A. Kline, V. Thomas, L. Abrahams, G. Hale & H. Waldmann: Immune reconstitution after allogeneic bone marrow transplantation depleted of T cells. *Transplantation* 69, 1341-1347 (2000)
185. Leskinen R: CD4 and CD8 expression of large granular lymphocytes after bone marrow transplantation and its effect on lymphocyte CD4/CD8 ratio. *Apmis* 101, 149-153 (1993)
186. Rocha B, P. Vassalli & D. Guy-Grand: Thymic and extrathymic origins of gut intraepithelial lymphocyte populations in mice. *J Exp Med* 180, 681-686 (1994)
187. Dejbakhsh-Jones S, L. Jerabek, I. L. Weissman & S. Strober: Extrathymic maturation of alpha beta T cells from hemopoietic stem cells. *J Immunol* 155, 3338-3344 (1995)
188. Mackall C. L, T. A. Fleisher, M. R. Brown, I. T. Magrath, A. T. Shad, M. E. Horowitz, L. H. Wexler, M. A. Adde, L. L. McClure & R. E. Gress: Lymphocyte depletion during treatment with intensive chemotherapy for cancer. *Blood* 84, 2221-2228 (1994)
189. Sprent J & C. D. Surh: Cytokines and T cell homeostasis. *Immunol Lett* 85, 145-149 (2003)
190. Surh C. D & J. Sprent: Homeostatic T cell proliferation: how far can T cells be activated to self-ligands? *J Exp Med* 192, F9-F14 (2000)
191. Freitas A. A & B. Rocha: Population biology of lymphocytes: the flight for survival. *Annu Rev Immunol* 18, 83-111 (2000)
192. Goldrath A. W: Maintaining the status quo: T-cell homeostasis. *Microbes Infect* 4, 539-545 (2002)
193. Gleeson P. A, B. H. Toh & I. R. van Driel: Organ-specific autoimmunity induced by lymphopenia. *Immunol Rev* 149, 97-125 (1996)
194. Coles A. J, M. Wing, S. Smith, F. Coraddu, S. Greer, C. Taylor, A. Weetman, G. Hale, V. K. Chatterjee, H. Waldmann & A. Compston: Pulsed monoclonal antibody treatment and autoimmune thyroid disease in multiple sclerosis. *Lancet* 354, 1691-1695 (1999)
195. Penhale W. J, A. Farmer & W. J. Irvine: Thyroiditis in T cell-depleted rats. Influence of strain, radiation dose, adjuvants and antilymphocyte serum. *Clin Exp Immunol* 21, 362-375 (1975)
196. Lesage S & C. C. Goodnow: Organ-specific autoimmune disease: a deficiency of tolerogenic stimulation. *J Exp Med* 194, F31-36 (2001)
197. Jendro M. C, T. Ganten, E. L. Matteson, C. M. Weyand & J. J. Goronzy: Emergence of oligoclonal T cell populations following therapeutic T cell depletion in rheumatoid arthritis. *Arthritis Rheum* 38, 1242-1251 (1995)
198. Mackall C. L, T. A. Fleisher, M. R. Brown, M. P. Andrich, C. C. Chen, I. M. Feuerstein, I. T. Magrath, L. H. Wexler, D. S. Dimitrov & R. E. Gress: Distinctions between CD8<sup>+</sup> and CD4<sup>+</sup> T-cell regenerative pathways result in prolonged T-cell subset imbalance after intensive chemotherapy. *Blood* 89, 3700-3707 (1997)
199. Mackall C. L, F. T. Hakim & R. E. Gress: T-cell regeneration: all repertoires are not created equal. *Immunol Today* 18, 245-251 (1997)
200. Mackall C. L & R. E. Gress: Pathways of T-cell regeneration in mice and humans: implications for bone marrow transplantation and immunotherapy. *Immunol Rev* 157, 61-72 (1997)
201. Freitas A. A & B. B. Rocha: Lymphocyte lifespans: homeostasis, selection and competition. *Immunol Today* 14, 25-29 (1993)
202. Freitas A. A & B. Rocha: Lymphocyte survival: a red queen hypothesis. *Science* 277, 1950 (1997)
203. Rocha B, N. Dautigny & P. Pereira: Peripheral T lymphocytes: expansion potential and homeostatic regulation of pool sizes and CD4/CD8 ratios in vivo. *Eur J Immunol* 19, 905-911 (1989)
204. Tanchot C, H. V. Fernandes & B. Rocha: The organization of mature T-cell pools. *Philos Trans R Soc Lond B Biol Sci* 355, 323-328 (2000)
205. L. Carroll: Alice in Wonderland. W.W. Norton and Co, NY (1992)
206. La Gruta N. L, I. R. Driel & P. A. Gleeson: Peripheral T cell expansion in lymphopenic mice results in a restricted T cell repertoire. *Eur J Immunol* 30, 3380-3386 (2000)
207. Koetz K, E. Bryl, K. Spickschen, W. M. O'Fallon, J. J. Goronzy & C. M. Weyand: T cell homeostasis in patients with rheumatoid arthritis. *Proc Natl Acad Sci U S A* 97, 9203-9208 (2000)
208. Ku C. C, M. Murakami, A. Sakamoto, J. Kappler & P. Marrack: Control of homeostasis of CD8<sup>+</sup> memory T cells by opposing cytokines. *Science* 288, 675-678 (2000)
209. Mackall C. L, T. J. Fry, C. Bare, P. Morgan, A. Galbraith & R. E. Gress: IL-7 increases both thymic-dependent and thymic-independent T-cell regeneration after bone marrow transplantation. *Blood* 97, 1491-1497 (2001)
210. Billingham R. E, L. Brent & P. B. Medawar: Actively acquired tolerance of foreign cells. *Nature* 172, 603 (1953)
211. Salama A. D, G. Remuzzi, W. E. Harmon & M. H. Sayegh: Challenges to achieving clinical transplantation tolerance. *J Clin Invest* 108, 943-948 (2001)
212. Halloran P. F: Immunosuppression in the post-adaptation period. *Transplantation* 70, 3-5 (2000)
213. Halwani F, R. D. Guttman, H. Ste-Croix & G. J. Prud'homme: Identification of natural suppressor cells in

- long-term renal allograft recipients. *Transplantation* 54, 973-977 (1992)
214. Reipert B, C. Scheuch, A. Lukowsky, P. Reinke, E. Fietze, W. D. Docke, G. Staffa, S. Czerlinski, R. Hetzer & H. D. Volk: CD3+ CD57+ lymphocytes are not likely to be involved in antigen-specific rejection processes in long-term allograft recipients. *Clin Exp Immunol* 89, 143-147 (1992)
215. Legendre C. M, R. D. Forbes, R. Loertscher & R. D. Guttman: CD4+/Leu-7+ large granular lymphocytes in long-term renal allograft recipients. A subset of atypical T cells. *Transplantation* 47, 964-971 (1989)
216. Leroy E, C. F. Calvo, M. Divine, M. F. Gourdin, F. Baujean, M. H. Ben Aribia, Z. Mishal, J. P. Vernant, J. P. Farcet & A. Senik: Persistence of T8+/HNK-1+ suppressor lymphocytes in the blood of long-term surviving patients after allogeneic bone marrow transplantation. *J Immunol* 137, 2180-2189 (1986)
217. Wang E. C & L. K. Borysiewicz: The role of CD8+, CD57+ cells in human cytomegalovirus and other viral infections. *Scand J Infect Dis Suppl* 99, 69-77 (1995)
218. Wang E. C, J. Taylor-Wiedeman, P. Perera, J. Fisher & L. K. Borysiewicz: Subsets of CD8+, CD57+ cells in normal, healthy individuals: correlations with human cytomegalovirus (HCMV) carrier status, phenotypic and functional analyses. *Clin Exp Immunol* 94, 297-305 (1993)
219. Ode-Hakim S, F. Kern, E. Fietze, S. Prosch, R. Ewert, H. Nügel, H. D. Volk & P. Reinke: Association between CMV-related graft injury and circulating cytokine-producing CD8+ memory cells. *Transplant Proc* 27, 951-953 (1995)
220. Dolstra H, F. Preijers, E. Van de Wiel-van Kemenade, A. Schattenberg, J. Galama & T. de Witte: Expansion of CD8+CD57+ T cells after allogeneic BMT is related with a low incidence of relapse and with cytomegalovirus infection. *Br J Haematol* 90, 300-307 (1995)
221. Labalette M, F. Salez, F. R. Pruvot, C. Noel & J. P. Dessaint: CD8 lymphocytosis in primary cytomegalovirus (CMV) infection of allograft recipients: expansion of an uncommon CD8+ CD57- subset and its progressive replacement by CD8+ CD57+ T cells. *Clin Exp Immunol* 95, 465-471 (1994)
222. Gratama J. W, H. C. Kluin-Nelemans, R. A. Langelaar, G. J. den Ottolander, T. Stijnen, J. D'Amaro, R. Torensma & H. J. Tanke: Flow cytometric and morphologic studies of HNK1+ (Leu 7+) lymphocytes in relation to cytomegalovirus carrier status. *Clin Exp Immunol* 74, 190-195 (1988)
223. Kern F, S. Ode-Hakim, K. Vogt, C. Hoflich, P. Reinke & H. D. Volk: The enigma of CD57+CD28- T cell expansion--anergy or activation? *Clin Exp Immunol* 104, 180-184 (1996)
224. Hazzan M, M. Labalette, C. Noel, G. Lelievre & J. P. Dessaint: Recall response to cytomegalovirus in allograft recipients: mobilization of CD57+, CD28+ cells before expansion of CD57+, CD28- cells within the CD8+ T lymphocyte compartment. *Transplantation* 63, 693-698 (1997)
225. Maher P, C. M. O'Toole, T. G. Wreghitt, D. J. Spiegelhalter & T. A. English: Cytomegalovirus infection in cardiac transplant recipients associated with chronic T cell subset ratio inversion with expansion of a Leu-7+ TS-C+ subset. *Clin Exp Immunol* 62, 515-524 (1985)
226. Dupuy d'Angéac A, S. Monier, C. Jorgensen, Q. Gao, A. Travaglio-Encinoza, C. Bologna, B. Combe, J. Sany & T. Reme: Increased percentage of CD3+, CD57+ lymphocytes in patients with rheumatoid arthritis. Correlation with duration of disease. *Arthritis Rheum* 36, 608-612 (1993)
227. Burns C. M, V. Tsai & N. J. Zvaifler: High percentage of CD8+, Leu-7+ cells in rheumatoid arthritis synovial fluid. *Arthritis Rheum* 35, 865-873 (1992)
228. Deem R. L, S. R. Targan, A. Niederlehner & F. Shanahan: The CD8+ Leu-7+ subset of T cells in Crohn's disease: distinction between cytotoxic and covert suppressor functions. *Clin Exp Immunol* 80, 387-394 (1990)
229. Vingerhoets J. H, G. L. Vanham, L. L. Kestens, G. G. Penne, R. L. Colebunders, M. J. Vandenbruaene, J. Goeman, P. L. Gigase, M. De Boer & J. L. Ceuppens: Increased cytolytic T lymphocyte activity and decreased B7 responsiveness are associated with CD28 down-regulation on CD8+ T cells from HIV-infected subjects. *Clin Exp Immunol* 100, 425-433 (1995)
230. Sadat-Sowti B, P. Debre, L. Mollet, L. Quint, F. Hadida, V. Leblond, G. Bismuth & B. Autran: An inhibitor of cytotoxic functions produced by CD8+CD57+ T lymphocytes from patients suffering from AIDS and immunosuppressed bone marrow recipients. *Eur J Immunol* 24, 2882-2888 (1994)
231. Mueller T. F, D. Gicklhorn, T. Jungraithmayr, M. Eickmann, H. Lange, K. Radsak & M. Reschke: Pattern and persistence of the epitope-specific IgM response against human cytomegalovirus in renal transplant patients. *J Clin Virol* 24, 45-56 (2002)
232. Jungraithmayr T. C, M. Reschke, S. O. Grebe, H. Lange, K. Radsak & T. F. Mueller: Assessment of cytomegalovirus infections using neopterin and a new immunoblot. *Clin Chim Acta* 310, 63-69 (2001)
233. Morley J. K, F. M. Batliwalla, R. Hingorani & P. K. Gregersen: Oligoclonal CD8+ T cells are preferentially expanded in the CD57+ subset. *J Immunol* 154, 6182-6190 (1995)
234. Azuma M, J. H. Phillips & L. L. Lanier: CD28- T lymphocytes. Antigenic and functional properties. *J Immunol* 150, 1147-1159 (1993)
235. Dupuy d'Angéac A, S. Monier, D. Pilling, A. Travaglio-Encinoza, T. Reme & M. Salmon: CD57+ T lymphocytes are derived from CD57- precursors by differentiation occurring in late immune responses. *Eur J Immunol*, 1503-1511 (1994)
236. Quan C. P, S. Watanabe, F. Vuillier, R. Pires, T. Matsuo, M. Stanislawski, J. Pillot & J. P. Bouvet: Purification and partial amino acid sequence of suppressive lymphokine from a CD8+ CD57+ human T hybridoma. *Immunology* 78, 205-209 (1993)
237. Maki T, H. Okazaki, M. L. Wood & A. P. Monaco: Suppressor cells in mice bearing intact skin allografts after blood transfusions. *Transplantation* 32, 463-466 (1981)
238. Hebib C, E. Leroy, M. Rouleau, S. Fornairon, D. Metivier, F. Hirsch, G. Kroemer, C. Legendre, A. Senik & B. Charpentier: Pattern of cytokine expression in circulation CD57+ T cells from long-term renal allograft recipients. *Transpl Immunol* 6, 39-47 (1998)
239. Shevach E. M, R. S. McHugh, A. M. Thornton, C. Piccirillo, K. Natarajan & D. H. Margulies: Control of autoimmunity by regulatory T cells. *Adv Exp Med Biol* 490, 21-32 (2001)
240. Sakaguchi S: Policing the regulators. *Nat Immunol* 2, 283-284 (2001)
241. Chang C. C, R. Ciobotariu, J. S. Manavalan, J. Yuan, A. I. Colovai, F. Piazza, S. Lederman, M. Colonna, R. Cortesini, R.

- Dalla-Favera & N. Suci-Foca: Tolerization of dendritic cells by TS cells: the crucial role of inhibitory receptors ILT3 and ILT4. *Nat Immunol* 28, 28 (2002)
242. Colovai A. I, M. Mirza, G. Vlad, S. Wang, E. Ho, R. Cortesini & N. Suci-Foca: Regulatory CD8+CD28- T cells in heart transplant recipients. *Hum Immunol* 64, 31-37 (2003)
243. Lloyd T. E, L. Yang, D. N. Tang, T. Bennett, W. Schober & D. E. Lewis: Regulation of CD28 costimulation in human CD8+ T cells. *J Immunol* 158, 1551-1558 (1997)
244. Linsley P. S, J. Bradshaw, M. Urnes, L. Grosmaire & J. A. Ledbetter: CD28 engagement by B7/BB-1 induces transient down-regulation of CD28 synthesis and prolonged unresponsiveness to CD28 signaling. *J Immunol* 150, 3161-3169 (1993)
245. Lake R. A, R. E. O'Hehir, A. Verhoef & J. R. Lamb: CD28 mRNA rapidly decays when activated T cells are functionally anergized with specific peptide. *Int Immunol* 5, 461-466 (1993)
246. Monteiro J, F. Batliwalla, H. Ostrer & P. K. Gregersen: Shortened telomeres in clonally expanded CD28-CD8+ T cells imply a replicative history that is distinct from their CD28+CD8+ counterparts. *J Immunol* 156, 3587-3590 (1996)
247. Rufer N, T. H. Brummendorf, B. Chapuis, C. Helg, P. M. Lansdorp & E. Roosnek: Accelerated telomere shortening in hematological lineages is limited to the first year following stem cell transplantation. *Blood* 97, 575-577 (2001)
248. Mathioudakis G, R. Storb, P. A. McSweeney, B. Torok-Storb, P. M. Lansdorp, T. H. Brummendorf, M. J. Gass, E. M. Bryant, J. Storek, M. E. Flowers, T. Gooley & R. A. Nash: Polyclonal hematopoiesis with variable telomere shortening in human long-term allogeneic marrow graft recipients. *Blood* 96, 3991-3994 (2000)
249. Wynn R. F, M. A. Cross, C. Hatton, A. M. Will, L. S. Lashford, T. M. Dexter & N. G. Testa: Accelerated telomere shortening in young recipients of allogeneic bone-marrow transplants. *Lancet* 351, 178-181 (1998)
250. Harley C. B, A. B. Futcher & C. W. Greider: Telomeres shorten during ageing of human fibroblasts. *Nature* 345, 458-460 (1990)
251. Effros R. B & G. Pawelec: Replicative senescence of T cells: does the Hayflick Limit lead to immune exhaustion? *Immunol Today* 18, 450-454 (1997)
252. Brenchley J. M, N. J. Karandikar, M. R. Betts, D. R. Ambrozak, B. J. Hill, L. E. Crotty, J. P. Casazza, J. Kuruppu, S. A. Migueles, M. Connors, M. Roederer, D. C. Douek & R. A. Koup: Expression of CD57 defines replicative senescence and antigen-induced apoptotic death of CD8+ T cells. *Blood* 14, 14 (2002)
253. Spaulding C, W. Guo & R. B. Effros: Resistance to apoptosis in human CD8+ T cells that reach replicative senescence after multiple rounds of antigen-specific proliferation. *Exp Gerontol* 34, 633-644 (1999)
254. Halloran P. F, A. Melk & C. Barth: Rethinking chronic allograft nephropathy: the concept of accelerated senescence. *J Am Soc Nephrol* 10, 167-181 (1999)
255. Weyand C. M & J. J. Goronzy: Premature immunosenescence in rheumatoid arthritis. *J Rheumatol* 29, 1141-1146 (2002)
256. Grossman Z, M. Meier-Schellersheim, A. E. Sousa, R. M. Victorino & W. E. Paul: CD4+ T-cell depletion in HIV infection: are we closer to understanding the cause? *Nat Med* 8, 319-323 (2002)
257. Ponchel F, A. W. Morgan, S. J. Bingham, M. Quinn, M. Buch, R. J. Verburg, J. Henwood, S. H. Douglas, A. Masurel, P. Conaghan, M. Gesinde, J. Taylor, A. F. Markham, P. Emery, J. M. van Laar & J. D. Isaacs: Dysregulated lymphocyte proliferation and differentiation in patients with rheumatoid arthritis. *Blood* 100, 4550-4556 (2002)
258. Meier-Kriesche H. U, A. O. Ojo, D. M. Cibrik, J. A. Hanson, A. B. Leichtman, J. C. Magee, F. K. Port & B. Kaplan: Relationship of recipient age and development of chronic allograft failure. *Transplantation* 70, 306-310 (2000)
259. S. P. Takemoto & P. Terasaki: Donor and recipient age. In: Clinical Transplants. Ed: Terasaki P. I, UCLA Tissue Typing Laboratory, Los Angeles (1988)
260. Velez R. L, K. R. Brinker, P. J. Vergne-Marini, D. A. Nesser, D. L. Long, G. Trevino, R. M. Dickerman & G. B. Helfrich: Renal transplantation with cyclosporine in the elderly population. *Transplant Proc* 23, 1749-1752 (1991)
261. Storek J, R. P. Witherspoon, R. Storb R: T cell reconstitution after bone marrow transplantation into adult patients does not resemble T cell development in early life. *Bone Marrow Transplant* 16, 413-425 (1995)
262. Gorski J, M. Yassai, X. Zhu, B. Kissela, C. Keever, N. Flomenberg: Circulating T cell repertoire complexity in normal individuals and bone marrow recipients analyzed by CDR3 size spectratyping. Correlation with immune status. *J Immunol* 152, 5109-5119 (1994)
263. Gaschet J, C. Denis, N. Milpied, M. M. Hallet, F. Romagne, A. Necker, R. Vivien, J. David-Ameline, F. Davodeau, M. Bonneville: Alterations of T cell repertoire after bone marrow transplantation: characterization of over-represented subsets. *Bone Marrow Transplant* 16, 427-435 (1995)
264. Masuko K, S. Kato, M. Hagihara, F. Tsuchida, Y. Takemoto, K. Izawa, T. Kato, S. Yamamori, Y. Mizushima, K. Nishioka, K. Tsuji, K. Yamamoto: Stable clonal expansion of T cells induced by bone marrow transplantation. *Blood* 87, 789-799 (1996)

**Abbreviations,** CD, cluster of differentiation; HIV, human immunodeficiency virus; RA, rheumatoid arthritis; TCR, T cell receptor; NK, natural killer; MHC, major histocompatibility complex; Syn, synonym; CMV, cytomegalovirus; CDC, complement-dependent lysis; ADCC, antibody-dependent cell cytotoxicity; MHC, major histocompatibility complex

**Key Words:** Immune Phenotypes, Lymphocyte Subsets, Homeostasis, Proliferation, Immunosuppression, Senescence, Transplantation, Regeneration, T Cells, Review

**Send correspondence to:** Thomas F. Mueller, MD, Department of Medicine, Renal Division, Molecular Immunology - Immunogenetics, Brigham and Women's Hospital, Harvard Medical School, 75 Francis St, Boston MA 02115, Tel: 617-732-5252, Fax: 617-264-6870, E-mail: tmueller@rics.bwh.harvard.edu