

Edited by:



Immune Engineered Solutions Scientific Advisory Board.
Chair: Pr Dominique Charron, MD, PhD (Paris)

Autologous White Blood Cells transfusion : toward a younger Immunity

by : Dominique J. CHARRON - MD, PhD

Professor of Immunology, Paris Diderot University
Head of Immunology and Translational Research Inserm/IUH, Hôpital Saint Louis (Paris,
France)

dominique.charron@sls.aphp.fr

With contributions from:

- Pr Michel SADELAIN, MD, PhD - Memorial Sloan Kettering Cancer Center, NY (USA)
- Pr Marc E.K. WEKSLER, MD, PhD - Cornell University, NY (USA)
- Dr Michèle GOODHARDT, PhD - INSERM and Hôpital Saint Louis, Paris (France)
- Dr Claude-Alain CUDENNEC, PhD - CSO Sankhia
- Dr Robert KELLER, MD, BioBancUSA

CONTENTS

I-	Introduction: Blood cells as medicine	p 3
II-	Immune functions are achieved by White Blood Cells	p 8
III-	Immune competence is eroded during aging	p 10
	- B lymphocytes	p 11
	- T lymphocytes	p 12
	1. CD4+ memory T cells,	p 12
	2. CD8+ memory T cells,	p 12
	3. Repertoire diversity,	p 13
	4. Regulatory T cells.	p 14
	- Monocytes, macrophages and dendritic cells	p 15
IV-	Cryopreservation of immune cells, a routine procedure	p 16
V-	Therapeutic benefit associated with autologous immune cell infusion	p 18
VI-	Conclusion / Perspective	p 22

Introduction : Blood cells as medicine

Blood and its various components, red blood cells (RBC), serum, plasma and protein derived products have been major providers of discovery in human biology and contributed to unprecedented medical development in the XXth Century. The most developed substitutive therapy is red blood cell transfusion, which is now commonly used and has proved to be highly beneficial on a worldwide basis. It has had a major impact in medicine and health care and has led to new logistics and public health policies, both national and international (1).

For the first time, a biological product obtained from one individual could be used as a therapeutic tool in unrelated individuals, hence breaking the “allogenic” barrier. This medical revolution was made possible by the discovery of the red blood cells ABO genetic system, allowing typing and matching of the product with the patient in order to avoid adverse transfusion reactions (2).

RBC transfusion also paved the way for deferred use of a biological product following storage, based on what was known about the life span of RBC in vitro.

In the wake of the worldwide success of RBC transfusion, however, the other major cellular component of blood, the white blood cells (WBC), were forgotten. Not only was their colour less visible, their concentration in blood lower, but also their genetic complexity, a very early observation, precluded the successful transfer of WBC between unrelated individuals.

The discovery of the HLA system (the major genetic system of WBC) (3) and the ever growing knowledge of HLA allelic diversity confirmed the idea that safe transfusion of WBC between unrelated individuals would be impossible. Interestingly, it was the febrile reactions due to the WBC contamination during red cell transfusion that led to the original identification of the HLA system. Therefore these WBC were considered as “evil” for RBC transfusion and blood transfusers focused on the development of procedures to eliminate contaminating WBC in RBC units (4). Moreover, the finding that WBC were the major reservoir of life-threatening viruses, such as HIV, hepatitis, HTLV, obviously meant that WBC had to be discarded for safety reasons. These procedures were very successful and improved the safety of RBC transfusion to current very high levels (5).

The combined effect of the extreme complexity of WBC biological genetic system and the iatrogenic risk of allogenic transfusion transmitted diseases prevented WBC being viewed as a useful biomedical resource. The risk of infection has now been almost completely eliminated in developed countries by donor selection and product safety procedures. Nevertheless, only a change in paradigm could lead to a change in opinion regarding the use of WBC for human therapy.

Although timid and scarce, attempts to use WBC in transfusion nevertheless occurred in the 70's. Granulocyte transfusion from allogenic donors was used to fight infections in aplastic

patients (after chemotherapy particularly in leukaemia) (6). The allogenic nature of these transfusions led to the expected allo-immunization, so that programs to select donors were developed to prevent or avoid this. Such schemes are used nowadays in the clinic for platelet transfusion as well and are very efficient (7).

Since the HLA barrier remains so “uncrossable” without major immunosuppressive therapy, it seems both logical and timely to re-explore autologous procedures. A new area of interest emerged with the identification of haematopoietic stem cells (HSC). These pluripotent non-differentiated cells are seen as a possible autologous biomedical tool capable of restoring the different blood cell lineages. Autologous Bone Marrow Transplantation (ABMT) was also used to overcome the aplasia induced by high dose chemotherapy (8). Initially, emphasis was only on haematopoietic reconstitution (myeloid), since it is the major first line concern for recovery of the patient, as it is the case in allogenic bone marrow transplantation.

Data on *immune reconstitution* and how to improve it after HSC transfer are relatively new and the tools to investigate post-graft immune dysfunction have only recently becoming available (9). Clearly, transplantation medicine is now aiming at enhancing the immune response to significantly improve overall survival and quality of life of patients after HSC transfer.

A breakthrough in WBC adoptive immunotherapy was made in 1990 when DLI (Donor Lymphocyte Infusion) was introduced as a clinically effective treatment of relapsing leukaemia after HSCT (10). This adoptive immunotherapy, which validates the use of autologous transfer of immune cells, was first used and proved successful in treating relapsing chronic myeloid leukaemia (CML). The procedure was based on the transfusion of buffy coat cells (WBC) from the original donor of a transplanted CML that had relapsed (11). DLI was

instrumental in demonstrating that an anti-leukemic allogeneic graft versus leukaemia (GVL) effect was effective, together with the initial myeloablative treatment, when used prior to bone marrow transplantation (BMT). These clinical results provided the rationale for the development of non-myeloablative HSCT in which the primary anti -leukemic modality is the donor T cell (12).

T cells used in DLI regimens were either fresh or cryopreserved from the BMT donor noticeably after T cell depletion. DLI was extended to treat other haematological malignancies: acute lymphoblastic leukaemia (ALL) (13), Acute myeloblastic leukaemia (AML) and myelodysplasia, as well as multiple myeloma lymphoma (14) and Chronic lymphocytic leukaemia (CLL) (15, 16, 17). Life threatening viral infections occurring in T cell depleted BMT patients were subsequently treated by DLI in transplanted patients.

The donor lymphocytes used therapeutically can be considered as immunologically autologous, since the transplanted recipient is fully reconstituted with the haematopoietic immune system of the same donor and demonstrates the clinical efficacy of “autologous” DLI in restoring anti viral immunity leading to prevention and care (18, 19, 20). This was documented using adoptive transfer of T cell clones in cytomegalovirus (21, 22, 23, 24) and Epstein Barr virus infections (in this later setting also preventing the occurrence of EBV induced lymphoma). Interestingly, persistence of CMV specific T cell clones for over 12 weeks in the peripheral blood of the receiver has been reported (20, 21). We have learnt from DLI that these collected lymphocytes are functionally efficient. Furthermore, these data provide the rationale for banking T cells and using these “off the shelf” to treat patients with viral infection. Autologous T cells but also T cells sharing HLA antigens, haplotype and fully HLA matched were proposed, although alloimmunization remains a limiting factor. Therefore the *autologous* immunocompetent cell infusion appeared definitely to be the safest choice.

Blood derived cells principally T lymphocytes (25) and dendritic cells (DC) (26) cells are the major cell types used in development of cell therapy. The vast increase in the number of clinical trials currently using adoptive immunotherapy in cancer and/or infectious diseases, indicates that adoptive immunotherapy will become a common medical procedure –at least in treatment of cancer – in the next 5 to 10 years (27). Thus, the availability of autologous immune cells collected before the onset of the disease and reinfused when needed, could be extremely useful. In this editorial review we will explore new avenues of autologous cryo-preserved WBC as a bio-resource for ulterior therapy and the starting point of a certain type of anticipatory medicine.

Immune functions are achieved by WBCs

Circulating white blood cells contain all of the essential cells needed for establishing immunity against infectious pathogens (viruses, bacteria, parasites) and tumor cells. In addition to granulocytes, they include B lymphocytes, T lymphocytes, natural killer (NK) cells, monocytes and dendritic cells (3).

These distinct cell categories control different aspects of the immune system that lead the fight against the targeted pathogen, infected cell or tumor cell. T cells are comprised of subtypes that are involved in either helping effector/killer T cells (Th1 cells) or B cells (Th2 cells), or in preventing/limiting effector responses (regulatory T cells). Individuals lacking T cell function, as for example patients with congenital or acquired immune deficiencies, are highly susceptible to various infections (28), showing the important protective role of these cells. Several autoimmune conditions (29), including juvenile diabetes and multiple sclerosis, are secondary to the inappropriate activation of T cells, which aberrantly turns against normal tissues, a type of immune response that is inhibited in a normal immune system.

T cells are essential for the elimination of virus-infected cells and tumor cells. It is well established that the absence of T cells or the presence of dysfunctional T cells leads to the inability to control common viruses (30) (e.g., EBV), greater disease morbidity or mortality (e.g., influenza) (31) or persistence of infection (e.g., chronic hepatitis) (32). In the case of cancer, it is now well established that tumors arising in mice lacking T cells or mice bearing T cells deficient in the capacity to secrete gamma-interferon, are more immunogenic and less aggressive when transferred to an immuno-competent mouse (33), demonstrating the positive role of the immune system in shaping the genetic profile and decreasing the malignancy of progressing tumors (34).

A central feature of the immune system is its ability to respond more rapidly and more efficiently to a subsequent challenge with a specific antigen/pathogen – a phenomenon known as immunological memory. This implies that the immune system is first educated and then retains memory, which leads to a better capacity to fight infections and to respond to vaccination leading to protective immunity. In this respect, the role of neutralizing antibodies is essential (3).

Dendritic cells, the most efficient professional antigen presenting cells, are paramount to jump start the T cell mediated immune response and benefit from NK cells stimuli to develop a robust innate immunity (35).

The scientific community has recently identified regulatory T cells and has started to dissect their complex role. These cells are potential interesting candidates for immunotherapy as illustrated by very recent experimental protocols designed for treating autoimmune diseases (36).

Any dysfunction in the cascade of cellular events involved in mounting an appropriate immune response against infectious agents or cancer cells is the origin of health threatening pathologies. These dysfunctions may arise as a result of either a pathogenic process, a viral infection or aging of the immune system.

Immune competence is eroded during aging

Aging has profound effects on the function of the immune system (37), leading to the enhanced susceptibility of the elderly to infections, increased cancer development and decreased response to immunization. Immunosenescence and how to overcome it or delay its development has become a major topic of basic and clinical immunology. Besides the enhanced susceptibility of the elderly to infections, their decreased response to immunization impairs their protection by vaccination.

Both antibody mediated (humoral) and cell mediated immunity are markedly affected by aging. It is well documented that the capacity of an individual to develop high affinity antibodies decrease with age, resulting in antibodies with lower titers and less functional particularly in neutralizing and opsonizing functions that are essential for protection from infections.

On the other hand immunological memory acquired during youth (ex. : CD4+ T cell derived from young naïve cells) functions well into old age, in contrast to the memory generated later in life (derived from older naïve cells) which functions less well (38).

Each category of immune cells contributes to immunosenescence.

- **B lymphocytes**

Even though thymic involution and diminished T lymphocyte production are the most striking changes in the elderly immune system (39), advanced aging is also accompanied by a decline in B lymphocyte production and consequently impaired humoral immune responses (40).

B lymphocytes are continuously generated in the bone marrow from pluripotent haematopoietic stem cells (HSC). Commitment to the B lineage and the subsequent differentiation of B cell progenitors depends on the co-ordinated expression of a series of transcription factors, including Ikaros, Pu1, EBF, E2A and Pax 5, as well as on the assembly of immunoglobulin genes (41). Antibody producing, immature B cells then exit to the periphery (spleen, lymph nodes), where they mature in response to antigenic stimulation.

A substantial change in all B cell compartments is observed with age. This defect starts at the stage of the HSC. Mouse models of aging have shown that although the numbers of immunophenotypic HSC do not decrease with age there is a decline in stem cell function (42, 43). Notably, HSC and early lymphoid progenitors progressively lose B cell potential with age and this loss is associated with decreased expression of the B cell transcription factors EBF and Pax5 (Maes et al submitted). A further age-related block occurs at the pro-B to pre-B cell transition, the stage at which immunoglobulin heavy chain gene rearrangement takes place, leading to a 4-fold reduction in pre-B cells and decreased generation of immature B cells (44, 45, 46). This in turn leads to diminished protective antibody responses with age, with lower titres of high affinity antigen-specific antibodies and decreased generation of memory B cells following vaccination. Decreased generation and through put of B cells in the bone marrow appears to be compensated by increased survival of peripheral B cell pools. These compensatory homeostatic processes lead to profound shifts in the antibody repertoire

and probably underlie the appearance of polyreactive and autoreactive antibodies observed with age (47, 48, 49).

- **T Lymphocytes**

- 1. CD4+ memory T cells.**

CD4+ T cells are essential in controlling the humoral responses (50) and the function of CD8+ T cells, both of which are key in the fight against infection. This has been widely demonstrated in animal models for CMV (51) infections and convincingly in humans particularly in the decreased efficacy of vaccination against influenza (52) and pneumococcal pneumonia in the elderly (53). It is clear that while CD4+ memory T cells generated in an aging individual persist in the periphery their ability to respond to a subsequent challenge to a given antigen is drastically impaired (54).

- 2. CD8+ memory T cells.**

The generation of protective CD8+ memory T cells relies on an adequate cognate function of CD4+ T cells during priming. If no proper help is delivered, the CD8+ memory T cells are defective upon antigenic recall and undergo activation induced apoptosis (55). While a stronger primary CD8+ response is seen in young animals following Lymphocytic ChorioMeningitis Virus infection there is no difference in the number of memory CD8+ T cells in aged mice (56). Moreover the function of these cells is drastically reduced upon re-stimulation (secondary response) in aged animal compared to young ones. This was also observed in humans. Age-related defects in memory CD8+ T cells function contributes to diminished protective effects of influenza vaccination in the elderly (57).

Protective immunity against tumors is less efficient with age, as observed in mice tumor models. Old mice implanted with a tumor do not develop protection to a tumor re-challenge while young mice do. This defect can then be overcome if the old animals are given extra co-stimulatory signals at priming, leading to augmentation of CD8+ T cells proliferation and increase cytotoxic T lymphocyte (CTL) activity (58).

Overall, age-related dysfunction of CD4+ and CD8+ memory T cells acts negatively both on humoral and cellular mediated responses. This explains why efficacy of new vaccination is diminished in the elderly. Interestingly however, one can predict that age-related diminished immune responses can be overcome by providing cells that were primed during youth.

Furthermore immunosenescence impacts not only on the function of T cells but also their diversity.

3. Repertoire diversity.

T cells are generated in the thymus, which they exit as “naïve” T cells. The sum of all the specificities arising through this process is referred to as the “repertoire” (each T cell bears one antigen receptor that is ultimately specific for one antigen). The production of naïve cells decreases through adulthood, resulting in shrinkage of the T cell repertoire (59). Also illustrative is the reduced T cell repertoire in patients recovering from an allogeneic bone marrow transplantation which is the cause of increased susceptibility to infection and mortality (60).

A diverse repertoire increases the probability of responding to any given antigen and the chance that more molecules deriving from a pathogen or a tumor cell will be recognized by

the immune system. This is not only pertinent at the time of the initial immune response, as elicited by vaccination, but later in the context of an on-going immune response (61).

The benefit of recruiting new naïve T cells to fight a chronic infection was recently demonstrated in murine experiments (62). While not demonstrated yet, it is reasonable to assume that the same holds true to fight cancer. Consequently, one can expect that any therapy designed to support and partially restore a diminished repertoire will increase the chances of mounting an appropriate immune reaction (63).

Overall, immunosenescence makes WBC a valuable biological resource. Younger WBC have unique “biological” quality with high potential preventative and curative clinical impact. Intrinsic molecular and metabolic alterations in signal transduction (64), glycosylation patterns in cytoskeleton and membrane lipid composition and dynamics have been observed in aged T cells compared to young ones (65, 66). It is therefore preferable to use young T cells rather than older ones that display an intrinsic immune hypo-responsiveness when adoptive T cell therapy is considered. It is particularly true in the course of cancer treatment, where T cells might be not only old but also may have been altered by the anticancer therapy (chemotherapy/irradiation).

4. Regulatory T cells.

The recent knowledge accumulated about the impact of aging and diseases upon regulatory T cells will soon be translated into the therapeutical field, for instance in autoimmune diseases (67, 68, 69).

- **Monocytes / macrophages /dendritic cells**

The monocyte/dendritic cells immune compartment is also affected by aging (70). As it has been observed in *ex vivo* models the neutrophils ability to phagocyte yeast and bacteria has been shown to be compromised in healthy elderly humans (71).

In addition Toll like receptors (TLR) ligands produced by antigen presenting cells (APC) to induce cytokine (particularly TNF α /IL6) production is decreased (72). The subsequent CD80 antigen upregulation is diminished and delayed. TLR1-2 function and expression are affected in APC (73). This may contribute to the impaired vaccine response and infection morbidity. As the most potent antigen presenting cells, dendritic cells (DC) play a central role in the early response to antigenic stimulation. It is important to emphasize that aging DC revealed a reduced phagocytosis capacity along with an increased secretion of pro-inflammatory cytokines after LPS stimulation (70). This may contribute to the well-described lower DC mediated T cell antigen proliferation capacity. These DC defects occurring with age are critical because dendritic cells are major effector cells in immuno-therapeutical developments (74).

Cryopreservation of immune cells, a routine procedure

Maintaining cells alive outside of the body was an early development in cell biology and subsequently cell cultures techniques have improved during the 20th century to become routine procedures. Indeed, blood cells were among the first cells to be cultured *ex vivo* and today, the isolation and maintenance of viable blood cells outside of the body are performed using well-established procedures (75).

Routinely, following blood donation (allogenic) plasma is aspirated and industrially processed, for manufacture of therapeutically useful factors, while the red blood cells are concentrated by centrifugation, typed for blood group and stored for later transfusion. During this procedure white blood cells, can also very easily be collected and stored (76).

The procedure used for preserving the viability of cells outside of the body depends on the expected duration of the conservation. For short term (hours) conservation, cells can be kept in suspension in saline or plasma at 2 to 10°C. Medium term (days to weeks) requires incubation of cells at 37°C in a complex medium with or without growth factors. Long term (months to years) cells can only be preserved unchanged by reducing the metabolism of cells through lowering the temperature (freezing).

Any damage during the freezing process can be prevented by introducing in the medium in which the cells are suspended a cryoprotectant (77). Dimethylsulfoxide (DMSO) (10 %) is the standard cryoprotectant validated for human cells.

Lowering temperature while optimizing the survival rate of cells requires a fast lowering of temperature. It can only be achieved by injecting massive amount of cold. The conventional

way to do this is to use liquid nitrogen (-196°C), which is routinely available in hospitals. The freezing process has been automated and now gives excellent survival rates (circa 94 %) (78). Once properly frozen the cells can be kept unchanged for years. Some haematopoietic cells have been stored for long term (79) and led to successful recovery after thawing. In order to guaranty the quality of the processes and the safety of the use of preserved cells, guidelines and accreditations have been set up. In all industrialized countries specific regulations have been set up (for example FDA and AABB (American Association of Blood Banks) issue guidelines).

Operators, places, machines, tubings, procedures, storage, ... are governed by rules that dictate best practices (80). The units running the process are required to obtain dedicated accreditation to do so and are submitted to periodic audits. As a consequence every process dealing with the manipulation of blood is subjected to thorough inspection as to safety and efficacy.

Once stored at -196°C , structure, metabolism and function of the frozen white blood cells are immobilized and will only be revived by restoring to a temperature above 0°C .

When needed, the storage bag containing the preserved cells will be retrieved, packed in dry ice (-70°C) and shipped by courier. All these steps are governed by guidelines edited by regulatory bodies for the graft of bone marrow (81). Depending on the therapeutic protocol, the sample is either subjected to a procedure for direct infusion or to a cellular therapy process (cultivation, stimulation, expansion, priming, ...) Whatever the regime, white blood cell samples are handled following quality controlled procedure.

Since they are all validated for either allogenic or autologous use from bone marrow, peripheral blood or umbilical cord blood (CB) (82), these procedures can be readily applied to the collection, storage and release of autologous WBC.

Therapeutic benefit associated with autologous

immune cell infusion

T cell responses are “restricted” by the subject’s HLA molecules, which are very diverse and highly immunogenic. Consequently, T cells from a donor are very unlikely to share all of the same HLA molecules and are thus unlikely to work in the recipient (because the T cells are not restricted to the appropriate HLA molecules) and likely to be rejected (because the recipient will perceive these T cells as foreign and reject them). If the recipient is severely immuno-deficient, the donor T cells will attack the recipient for the same reason (this attack can be lethal, as in acute graft-versus-host disease) (3). This is why for white blood cells, autologous transfer is so essential, in contrast to red blood cells, for which unrelated donors can serve as a safe and effective source.

WBC contains autologous naïve T cells able to replenish the repertoire and therefore the chance of responding to any antigen and memory T cells, i.e., T cells that were previously stimulated by vaccination or earlier infection (59). As a consequence elicited T cell clones will expand more rapidly in response to a new antigenic exposure.

Vaccinations

As individuals age, their ability to respond to and eliminate pathogens decreases, leading to increased incidence and severity of infectious diseases. Furthermore the efficacy of vaccinations is frequently reduced in the elderly. Well-documented examples include influenza vaccine that has only 40-60% efficiency in older individuals (83).

The rates of complications, hospitalisation and death after influenza infection are then greater with advancing age, so that of the 30-50 thousand deaths due to influenza each year in the US, 90% are of persons aged 65 years and over (84). In addition to influenza, there is evidence of reduced responses with vaccinations for tetanus, encephalitis, hepatitis, and pneumonia in the elderly (85, 86, 87). Increased susceptibility to infections and reduced protection after vaccination reflect diminished protective antibody responses with age. Indeed there are lower titres of high affinity antigen-specific antibodies and decreased generation of memory B cells following vaccination in the elderly (88, 89). This in turn results from profound age-related changes in the immune system, including decreased generation of B cells and CD4+ T cell helper function (90, 91).

Cancer

Autologous WBC – especially T cells and dendritic cells – are increasingly used in adoptive anticancer immunotherapy (92, 93, 94).

Genetically redirected autologous T cells and in vitro stimulated autologous T cells may soon prove to be an effective means of targeting tumors (95). There are increasing numbers of reports on the efficiency of autologous procedures in variety of cancers: melanoma (96), non-Hodgkin's lymphoma (97), metastatic renal carcinoma (98), hepatocellular carcinoma (99), gastric cancer (100), ovarian carcinoma (101), glioma (102), head and neck cancer (103), ... Recent articles from US and European clinical scientists demonstrate that T cells harvested from a patient – at a time when immune cells no longer have the best responses – manipulated and expanded before being re-infused in the same individual displayed a clear cut capacity to target the cancer cells and mediate tumor regression. Moreover, these empowered cells have

been shown to remain circulating in the blood a long period of time following re-infusion and may therefore be able to continue immuno-surveillance long after autologous cell therapy (104).

Also NK cells have been shown to mediate anti-tumor responses in the context of allogeneic bone marrow transplantation, and may be used in autologous settings for instance in bladder cancer (105).

Therapeutic vaccination against cancer

Dendritic cells used in many vaccination strategies currently under development in phase I, II and III studies (106). Marketing authorization for such medical autologous processes are announced for the forthcoming year in the States to treat resistant prostatic cancers.

Moreover dendritic cells and B cells are often used to expand T cells that are subsequently infused for therapeutic purposes.

From a global survey of the literature from 1990 to date it has been shown (Sadelain, M. et al. submitted) a tremendous increase of reports dealing with the use of immune cells in anticancer trials. T cell subsets and dendritic cells have been confirmed as focusing the efforts to develop therapeutic protocols. Melanoma, renal carcinoma, lymphoma, leukaemia, gliomas are the most cited targeted cancer in these trials.

Infections and other diseases

Preliminary results have also been reported showing similar efficacy of autologous adoptive immunotherapy against infectious agents (Epstein-Barr virus) (107) and a variety of diseases such as rheumatoid arthritis (108), scleroderma (109), ...

In addition to the benefit derived from elicited responses of immune selected cells, there is a well established role of repopulating capacity of haematopoietic stem cells (HSC) circulating in the blood and present in the buffy coat. Their frequency in peripheral blood is inferior to that in the bone marrow, but they have been shown to home correctly to haematopoietic tissues and help restore the different blood cell lineages in patients frequently submitted to hematosuppressive regimens (chemotherapy, radiotherapy). A better hemato-restoring capacity has been identified in young cells (110).

Conclusion / Perspective

Autologous WBC as an unique bio-resource

Cryo-preserved autologous WBC can today be seen as a unique promising bio-resource. Bio-banking of WBC has now become a routine procedure with approved standards for collecting, freezing, storing and releasing the bio-product for DLI as well as CB transplantation. Industrial procedures are well developed, in use and are totally safe. There is world-wide consensus among the Scientific / Medical community as to the overall process used for storing WBC, which is strictly controlled by national and international regulatory bodies. The whole process of banking – releasing WBC has been fully validated by and is operational in Cord Blood Banks, including the organisation of worldwide delivery of the bio-product.

WBC cells contain all the necessary cells to fight infectious diseases and tumour development, hence their exceptional potential in therapy. Nevertheless, WBC function declines with age. Indeed, immunosenescence is well documented in humans and there is overwhelming data from animal models. While aging of the immune system starts early in adult life with the slow decline of thymic function, erosion of immune competence is a continuous stochastic process throughout adulthood. In order to boost vanishing immunity, young adult WBC are clearly more competent, educated and diverse as compared to umbilical CB cells (and Stem Cell derived cells).

Autologous WBC versus Umbilical Cord Blood Cells

Although the therapeutic value of CB cells is widely recognized, up to now they have only been used in an allogenic setting for haematopoietic reconstitution/recovery. CB cells are virgin with respect to immunity, unlike “experienced” adult WBC that have encountered numerous pathogens during childhood and adult life. Moreover CB collection is a “one time” opportunity at birth. If missed it is irretrievably lost. Cord stem cells are also quantitatively limited.

Autologous WBC versus Haematopoietic Stem Cells

While haematopoietic stem cells have the potential to generate all the blood cell lineages, reconstitution of WBC is long, taking over 1 year postgraft to generate a fully functional immune system. Furthermore, the immune cells generated are immunologically immature. Autologous WBC are immediately functional and immunologically experienced.

The memory of past encounters that only a mature immune system has kept is lacking within the SC derived lineages. So adult lymphocytes are clearly more suited than CB or HSC to boost immune function during periods of immunodepression.

Autologous stem cells from bone marrow or peripheral blood of a patient are an alternative therapeutic source of immune cells, especially as it was long thought that stem cells that have been quiescent for decades do not age. Recent evidence shows that this is clearly not the case, so that when possible the use of autologous stem cells stored when the patient was young is likely to be preferred in forthcoming regenerative medicine. These can be retrieved from an individual’s WBC preserved at a younger age. WBC may will turnout to a future bio-resource for autologous « young » stem cells.

Cryo-preserved autologous WBC in anti-cancer treatment

Several arguments are in favour of the usefulness of these cells in the fight against cancer. An optimal immune system is clearly a key success-factor in cancer immunotherapy. Unfortunately at the time when these anti-cancer and anti-micrometastase treatments (adoptive immunotherapy, vaccines) are given, the patient's immune system is far from being at it's best, either because development of the cancer has diminished the immune response, or because previous cancer treatments (chemo- / radio-therapy) have impaired immune integrity.

The availability of cryo-preserved autologous WBC will permit the use of therapeutic vaccination against cancer which is a far less toxic than current therapies. It will be especially useful in elderly patients that are particularly frail.

Vaccination has been shown to be more efficient in terms of controlling micro metastasis frequency and residual tumor cells. Moreover the infusion of young, healthy cells will help to restore efficient immune function in the host frequently submitted to a cytoreductive therapy.

Adoptive immunotherapy (AI) is developing very rapidly in oncology, for infections and chronic inflammatory diseases. Numerous innovative preclinical and early clinical trials have been initiated and favourable outcomes of phase III clinical trials have been already released.

In the next couple of years, it is probable that AI will be offered to a large population of patients as a validated therapy. Since AI relies on the patient's own immune cells, the availability of these cells at the right time and under optimal functional capacity will be the limiting factor for this therapy to be apply. The immuno-competent cells will be reinfused either directly or after expansion and/or genetic engineering processes according to the appropriate protocols.

Should the pathologic status of a patients indicate immune therapy, there will be a strong incentive to choose his own preserved immune cells - when available - for 3 decisive reasons:

These cells are younger. The greater diversity and broader immune repertoire of T and B lymphocytes observed in younger individuals insure that they are able to target, recognize and fight better a large number of pathogenic antigens, including tumor antigens.

These cells are healthier.

The use of these cells will avoid and circumvent the functional decline due to an accumulation of intrinsic metabolic damages which occur during the ageing process.

Theses cells are safer.

The risk of infusing contaminating agents or malignant metastatic circulating cells (including the very rare but deadly cancer stem cells) present in the blood cells when collected at the time of diagnosis will be eliminated by thawing cells cryopreserved several years before when the individual was disease free.

The availability of cryo-preserved fresh WBC will not only circumvent these hurdles but will also provide elderly patients with more vigorous immune response than their current immune cells can achieve. WBC transfusion may well impact health in the XXI th century to the same extent as RBC transfusion did over the XX th century

REFERENCES

1. Keeping Blood Transfusions Safe : FDA's Multi-layered Protections for Donated Blood. 2002. US Food and Drug Administration. Publication No. Fs 02-1.
2. *Petrides M, Stack, G.* 2001. Pratical Guide to Transfusion Medicine. AABB press, ISBN # 1-56395-128-2
3. *Mak T W, Saunders M E.* 2006. The Immune Response Basic and Clinical Principles. Chapter 27 : Transplantation pp 873-917. Elsevier Inc. Academic Press, ISBN-10 # 0-12-088451-8
4. *Ratko TA, Cummings JP, Oberman HA, Crookston KP, DeChristopher PJ, Eastlund DT, Godwin JE, Sacher RA, Yawn DH, Matuszewski KA; University Health System Consortium.* 2001. Evidence-based recommendations for the use of WBC-reduced cellular blood components. *Transfusion.* 41(10):1310-9.
5. *Luban NL.* 2005. Transfusion safety: Where are we today? *Ann N Y Acad Sci.* 1054:325-41.
6. *Lowenthal RM, Grossman L, Goldman JM, Storring RA, Buskard NA, Park DS, Murphy BC, Spiers AS, Galton DA.* 1975. Granulocyte transfusions in treatment of infections in patients with acute leukaemia and aplastic anaemia. *Lancet.* 1(7903):353-8.
7. *Heddle NM, Cook RJ, Sigouin C, Slichter SJ, Murphy M, Rebull P; BEST Collaborative (Biomedical Excellence for Safer Transfusion).* 2006. A descriptive analysis of international transfusion practice and bleeding outcomes in patients with acute leukemia. *Transfusion.* 46(6):903-11.
8. *Awedan AA.* 2002. High intensity regimens with autologous hematopoietic stem cell transplantation as treatment of multiple myeloma. *Ann Transplant.* 7(2):38-43.
9. *Porter DL, June CH.* 2005. T-cell reconstitution and expansion after hematopoietic stem cell transplantation: "T" it up! *Bone Marrow Transplant.* 35(10):935-42.
10. *Kolb HJ, Mittermuller J, Clemm C, Holler E, Ledderose G, Brehm G, Heim M, Wilmanns W.* 1990. Donor leukocyte transfusions for treatment of recurrent chronic myelogenous leukemia in marrow transplant patients. *Blood.* 76(12):2462-5.
11. *Luznik L, Fuchs EJ.* 2002 Donor lymphocyte infusions to treat hematologic malignancies in relapse after allogeneic blood or marrow transplantation. *Cancer Control.* 9(2):123-37.
12. *Kolb HJ, Schattenberg A, Goldman JM, Hertenstein B, Jacobsen N, Arcese W, Ljungman P, Ferrant A, Verdonck L, Niederwieser D, van Rhee F, Mittermueller J, de Witte T, Holler E, Ansari H; European Group for Blood and Marrow Transplantation Working Party Chronic Leukemia.* 1995. Graft-versus-leukemia effect of donor lymphocyte transfusions in marrow grafted patients. *Blood.* 86(5):2041-50.

13. *Collins RH Jr, Goldstein S, Giral S, Levine J, Porter D, Drobyski W, Barrett J, Johnson M, Kirk A, Horowitz M, Parker P.* 2000. Donor leukocyte infusions in acute lymphocytic leukemia. *Bone Marrow Transplant.* 26(5):511-6.
14. *Salama M, Nevill T, Marcellus D, Parker P, Johnson M, Kirk A, Porter D, Giral S, Levine JE, Drobyski W, Barrett AJ, Horowitz M, Collins RH.* 2000. Donor leukocyte infusions for multiple myeloma. *Bone Marrow Transplant.* 26(11):1179-84
15. *Mehta J, Powles R, Singhal S, Iveson T, Treleaven J, Catovsky D.* 1996. Clinical and hematologic response of chronic lymphocytic and prolymphocytic leukemia persisting after allogeneic bone marrow transplantation with the onset of acute graft-versus-host disease: possible role of graft-versus-leukemia. *Bone Marrow Transplant.* 17(3):371-5.
16. *deMagalhaes-Silverman M, Donnenberg A, Hammert L, Lister J, Myers D, Simpson J, Ball E.* 1997. Induction of graft-versus-leukemia effect in a patient with chronic lymphocytic leukemia. *Bone Marrow Transplant.* 20(2):175-7.
17. *Collins RH Jr, Shpilberg O, Drobyski WR, Porter DL, Giral S, Champlin R, Goodman SA, Wolff SN, Hu W, Verfaillie C, List A, Dalton W, Ognoskie N, Chetrit A, Antin JH, Nemunaitis J.* 1997. Donor leukocyte infusions in 140 patients with relapsed malignancy after allogeneic bone marrow transplantation. *J Clin Oncol.* 15(2):433-44.
18. *Hromas R, Cornetta K, Srour E, Blanke C, Broun ER.* 1994 Donor leukocyte infusion as therapy of life-threatening adenoviral infections after T-cell-depleted bone marrow transplantation. *Blood.* 84(5):1689-90.
19. *Kishi Y, Kami M, Oki Y, Kazuyama Y, Kawabata M, Miyakoshi S, Morinaga S, Suzuki R, Mori S, Muto Y.* 2000. Donor lymphocyte infusion for treatment of life-threatening respiratory syncytial virus infection following bone marrow transplantation. *Bone Marrow Transplant.* 26(5):573-6.
20. *Riddell SR, Watanabe KS, Goodrich JM, Li CR, Agha ME, Greenberg PD.* 1992. Restoration of viral immunity in immunodeficient humans by the adoptive transfer of T cell clones. *Science.* 257(5067):238-41.
21. *Walter EA, Greenberg PD, Gilbert MJ, Finch RJ, Watanabe KS, Thomas ED, Riddell SR.* 1995. Reconstitution of cellular immunity against cytomegalovirus in recipients of allogeneic bone marrow by transfer of T-cell clones from the donor. *N Engl J Med.* 333(16):1038-44.
22. *Papadopoulos EB, Ladanyi M, Emanuel D, Mackinnon S, Boulad F, Carabasi MH, Castro-Malaspina H, Childs BH, Gillio AP, Small TN, et al.* 1994. Infusions of donor leukocytes to treat Epstein-Barr virus-associated lymphoproliferative disorders after allogeneic bone marrow transplantation. *N Engl J Med.* 330(17):1185-91.
23. *Rooney CM, Smith CA, Ng CY, Loftin S, Li C, Krance RA, Brenner MK, Heslop HE.* 1995. Use of gene-modified virus-specific T lymphocytes to control Epstein-Barr-virus-related lymphoproliferation. *Lancet.* 345(8941):9-13.

24. *Rooney CM, Smith CA, Ng CY, Loftin SK, Sixbey JW, Gan Y, Srivastava DK, Bowman LC, Krance RA, Brenner MK, Heslop HE.* 1998. Infusion of cytotoxic T cells for the prevention and treatment of Epstein-Barr virus-induced lymphoma in allogeneic transplant recipients. *Blood.* 92(5):1549-55.
25. *Foster AE, Rooney CM.* 2006. Improving T cell therapy for cancer. *Expert Opin Biol Ther.* 6(3):215-29.
26. *Lin AM, Hershberg RM, Small EJ.* 2006. Immunotherapy for prostate cancer using prostatic acid phosphatase loaded antigen presenting cells. *Urol Oncol.* 24(5):434-41.
27. *Ferrantini M, Capone I, Marincola FM, Parmiani G, Belardelli F.* 2006. International Meeting "Immunotherapy of Cancer: Challenges and Needs". *Cancer Immunol Immunother.*
28. *Kiehn TE.* 1989. Bacteremia and fungemia in the immunocompromised patient. *Eur J Clin Microbiol Infect Dis.* 8(9):832-7.
29. *Berthelot JM, Maugars Y.* 2004. Role for suppressor T cells in the pathogenesis of autoimmune diseases (including rheumatoid arthritis). Facts and hypotheses. *Joint Bone Spine.* 71(5):374-80.
30. *Heemskerk B, van Vreeswijk T, Veltrop-Duits LA, Sombroek CC, Franken K, Verhoosel RM, Hiemstra PS, van Leeuwen D, Rensing ME, Toes RE, van Tol MJ, Schilham MW.* 2006. Adenovirus-specific CD4+ T cell clones recognizing endogenous antigen inhibit viral replication in vitro through cognate interaction. *J Immunol.* 177(12):8851-9.
31. *Wade JC.* 2006. Viral infections in patients with hematological malignancies. *Hematology Am Soc Hematol Educ Program.* :368-74.
32. *Huang L, Koziel MJ.* 2000. Immunology of hepatitis C virus infection. *Curr Opin Gastroenterol.* 16(6):558-564.
33. *Hollenbaugh JA, Dutton RW.* 2006. IFN-gamma regulates donor CD8 T cell expansion, migration, and leads to apoptosis of cells of a solid tumor. *J Immunol.* 177(5):3004-11.
34. *Dunn GP, Koebel CM, Schreiber RD.* 2006. Interferons, immunity and cancer immunoediting. *Nat Rev Immunol.* 6(11):836-48.
35. *Capobianco A, Rovere-Querini P, Rugarli C, Manfredi AA.* Melanoma cells interfere with the interaction of dendritic cells with NK/LAK cells. 2006. *Int J Cancer.* 119(12):2861-9.
36. *Tang Q, Bluestone JA.* 2006. Regulatory T-cell physiology and application to treat autoimmunity. *Immunol Rev.* 212:217-37.
37. *Hodes RJ.* 2005. Aging and the immune system. *Curr Opin Immunol.* 17(5):455-6
38. *Haynes L.* 2005. The effect of aging on cognate function and development of immune memory. *Curr Opin Immunol.* 17(5):476-9.

39. *Gruver A, Hudson L, Sempowski G.* 2007. Immunosenescence of ageing. *J Pathol.* 211(2):144-156
40. *Weksler ME, Goodhardt M, Szabo P.* 2002. The effect of age on B cell development and humoral immunity. *Springer Semin Immunopathol.* 24(1):35-52.
41. *Singh H, Pongubala JM.* 2006. Gene regulatory networks and the determination of lymphoid cell fates. *Curr Opin Immunol.* 18(2):116-20
42. *Morrison SJ, Wandycz AM, Akashi K, Globerson A, Weissman IL.* 1996. The aging of hematopoietic stem cells. *Nat Med.* 2(9):1011-6.
43. *Janzen V, Forkert R, Fleming HE, Saito Y, Waring MT, Dombkowski DM, Cheng T, DePinho RA, Sharpless NE, Scadden DT.* 2006 Stem-cell ageing modified by the cyclin-dependent kinase inhibitor p16INK4a. *Nature.* 443(7110):421-6.
44. *Stephan RP, Sanders VM, Witte PL.* 1996. Stage-specific alterations in murine B lymphopoiesis with age. *Int Immunol.* 8(4):509-18.
45. *Labrie JE 3rd, Sah AP, Allman DM, Cancro MP, Gerstein RM.* 2004. Bone marrow microenvironmental changes underlie reduced RAG-mediated recombination and B cell generation in aged mice. *J Exp Med.* 200(4):411-23.
46. *Li F, Jin F, Freitas A, Szabo P, Weksler ME.* 2001. Impaired regeneration of the peripheral B cell repertoire from bone marrow following lymphopenia in old mice. *Eur J Immunol.* 31(2):500-5.
47. *Szabo P, Li F, Mathew J, Lillvis J, Weksler ME.* 2004. Evolution of B-cell clonal expansions with age. *Cell Immunol.* 231(1-2):158-67.
48. *Weksler ME, Szabo P.* 2000. The effect of age on the B-cell repertoire. *J Clin Immunol.* 20(4):240-9.
49. *Cancro MP.* 2005. B cells and aging: gauging the interplay of generative, selective, and homeostatic events. *Immunol Rev.* 205:48-59.
50. *Amyes E, Hatton C, Montamat-Sicotte D, Gudgeon N, Rickinson AB, McMichael AJ, Callan MF.* 2003. Characterization of the CD4+ T cell response to Epstein-Barr virus during primary and persistent infection. *J Exp Med.* 198(6):903-11.
51. *McVoy MA, Adler SP.* 1989. Immunologic evidence for frequent age-related cytomegalovirus reactivation in seropositive immunocompetent individuals. *J Infect Dis.* 160(1):1-10.
52. *Morgan R, King D.* 1996. Influenza vaccination in the elderly. *Postgrad Med J.* 72(848):339-42
53. *Rivetti D, Jefferson T, Thomas R, Rudin M, Rivetti A, Di Pietrantonj C, Demicheli V.* 2006. Vaccines for preventing influenza in the elderly. *Cochrane Database Syst Rev.* 3:CD004876.

54. *Kapasi ZF, Murali-Krishna K, McRae ML, Ahmed R.* 2002. Defective generation but normal maintenance of memory T cells in old mice. *Eur J Immunol.* 32(6):1567-73.
55. *Janssen EM, Droin NM, Lemmens EE, Pinkoski MJ, Bensing SJ, Ehst BD, Griffith TS, Green DR, Schoenberger SP.* 2005. CD4+ T-cell help controls CD8+ T-cell memory via TRAIL-mediated activation-induced cell death. *Nature.* 434(7029):88-93.
56. *Homann D, Teyton L, Oldstone MB.* 2001. Differential regulation of antiviral T-cell immunity results in stable CD8+ but declining CD4+ T-cell memory. *Nat Med.* 7(8):913-9.
57. *Deng Y, Jing Y, Campbell AE, Gravenstein S.* 2004. Age-related impaired type 1 T cell responses to influenza: reduced activation ex vivo, decreased expansion in CTL culture in vitro, and blunted response to influenza vaccination in vivo in the elderly. *J Immunol.* 172(6):3437-46.
58. *Lustgarten J, Dominguez AL, Thoman M.* 2004. Aged mice develop protective antitumor immune responses with appropriate costimulation. *J Immunol.* 173(7):4510-5.
59. *Goronzy JJ, Weyand CM.* 2005. T cell development and receptor diversity during aging. *Curr Opin Immunol.* 17(5):468-75.
60. *Storek J, Joseph A, Espino G, Dawson MA, Douek DC, Sullivan KM, Flowers ME, Martin P, Mathioudakis G, Nash RA, Storb R, Appelbaum FR, Maloney DG.* 2001. Immunity of patients surviving 20 to 30 years after allogeneic or syngeneic bone marrow transplantation. *Blood.* 98(13):3505-12.
61. *Zinkernagel RM, Hengartner H.* 2004. On immunity against infections and vaccines: credo 2004. *Scand J Immunol.* 60(1-2):9-13.
62. *Vezyz V, Masopust D, Kemball CC, Barber DL, O'Mara LA, Larsen CP, Pearson TC, Ahmed R, Lukacher AE.* 2006. Continuous recruitment of naive T cells contributes to heterogeneity of antiviral CD8 T cells during persistent infection. *J Exp Med.* 203(10):2263-9.
63. *Talvensaari K, Clave E, Douay C, Rabian C, Garderet L, Busson M, Garnier F, Douek D, Gluckman E, Charron D, Toubert A.* 2002. A broad T-cell repertoire diversity and an efficient thymic function indicate a favorable long-term immune reconstitution after cord blood stem cell transplantation. *Blood.* 99(4):1458-64.
64. *Sadighi Akha AA, Miller RA.* 2005. Signal transduction in the aging immune system. *Curr Opin Immunol.* 17(5):486-91.
65. *Garcia GG, Miller RA.* 2002. Age-dependent defects in TCR-triggered cytoskeletal rearrangement in CD4+ T cells. *J Immunol.* 169(9):5021-7.
66. *Garcia GG, Berger SB, Sadighi Akha AA, Miller RA.* 2005. Age-associated changes in glycosylation of CD43 and CD45 on mouse CD4 T cells. *Eur J Immunol.* 35(2):622-31.

67. *Kohm AP, Carpentier PA, Anger HA, Miller SD.* 2002. Cutting edge: CD4+CD25+ regulatory T cells suppress antigen-specific autoreactive immune responses and central nervous system inflammation during active experimental autoimmune encephalomyelitis. *J Immunol.* 169(9):4712-6.
68. *Frey O, Petrow PK, Gajda M, Siegmund K, Huehn J, Scheffold A, Hamann A, Radbruch A, Brauer R.* 2005. The role of regulatory T cells in antigen-induced arthritis: aggravation of arthritis after depletion and amelioration after transfer of CD4+CD25+ T cells. *Arthritis Res Ther.* 7(2):R291-301.
69. *Kearley J, Barker JE, Robinson DS, Lloyd CM.* 2005. Resolution of airway inflammation and hyperreactivity after in vivo transfer of CD4+CD25+ regulatory T cells is interleukin 10 dependent. *J Exp Med.* 202(11):1539-47.
70. *Grolleau-Julius A, Garg MR, Mo R, StoolmanLL, Yung RL.*2006. Effect of aging on bone marrow-derived murine CD11c+CD4-CD8alpha- dendritic cell function. *J Gerontol A Biol Sci Med Sci.* 61(10):1039-47.
71. *Fulop T, Larbi A, Douziech N, Fortin C, Guerard KP, Lesur O, Khalil A, Dupuis G.* 2004. Signal transduction and functional changes in neutrophils with aging. *Aging Cell.* 3(4):217-26.
72. *Boehmer ED, Goral J, Faunce DE, Kovacs EJ.* 2004. Age-dependent decrease in Toll-like receptor 4-mediated proinflammatory cytokine production and mitogen-activated protein kinase expression. *J Leukoc Biol.* 75(2):342-9.
73. *Gomez CR, Boehmer ED, Kovacs EJ.* 2005. The aging innate immune system. *Curr Opin Immunol.* 17(5):457-62.
74. *Buchsel PC, DeMeyer ES.* 2006. Dendritic cells: emerging roles in tumor immunotherapy. *Clin J Oncol Nurs.* 10(5):629-40.
75. *Sacher R.* 2002. Cellular therapy : New frontiers in transfusion medicine. AABB Press ISBN # 1-56395-158-4.
76. *Wolf CE, Meyer M, Riggert J.* 2005. Leukapheresis for the extraction of monocytes and various lymphocyte subpopulations from peripheral blood: product quality and prediction of the yield using different harvest procedures. *Vox Sang.* 88(4):249-55.
77. *Celluzzi CM, Welbon C.* 2003. A simple cryopreservation method for dendritic cells and cells used in their derivation and functional assessment. *Transfusion.* 43(4):488-94.
78. *Dobrila L, Jiang S, Chapman J, Marr D, Kryston K, Rubinstein P.* 2006. ThermoGenesis AXP™ and BioArchive™ Systems for Automated Cord Blood Banking. 32nd Annual Meeting of the European Groups for Blood and Marrow Transplantation, Hamburg, Germany, March 19-22 2006.
79. *Donnenberg AD, Koch EK, Griffin DL, Stanczak HM, Kiss JE, Carlos TM, Buchbarker DM, Yeager AM.* 2002. Viability of cryopreserved BM progenitor cells stored for more than a decade. *Cytotherapy.* 4(2):157-63.

80. CFR Mini-Hanbook 2006. AABB press ISBM # 1-56395-231-9.
81. *Brecher, ME*. 2005. technical Manual. 15th Edition. AABB press ISBM # 1-56395-1967.
82. *Broxmeyer, HE*. 2004. Cord blood : Biology, immunology, Banking and clinical transplantation. AABB press ISBM # 1-56395-176-2.
83. *Vu T, Farish S, Jenkins M, Kelly H*. 2002. A meta-analysis of effectiveness of influenza vaccine in persons aged 65 years and over living in the community. *Vaccine*. 20(13-14):1831-6.
84. *Thompson WW, Shay DK, Weintraub E, Brammer L, Cox N, Anderson LJ, Fukuda K*. 2003. Mortality associated with influenza and respiratory syncytial virus in the United States. *JAMA*. 289(2):179-86.
85. *Cook JM, Gualde N, Hessel L, Mounier M, Michel JP, Denis F, Ratinaud MH*. 1987. Alterations in the human immune response to the hepatitis B vaccine among the elderly. *Cell Immunol*. 109(1):89-96.
86. *Hainz U, Jenewein B, Asch E, Pfeiffer KP, Berger P, Grubeck-Loebenstien B*. 2005. Insufficient protection for healthy elderly adults by tetanus and TBE vaccines. *Vaccine*. 23(25):3232-5.
87. *Musher DM, Chapman AJ, Goree A, Jonsson S, Briles D, Baughn RE*. 1986. Natural and vaccine-related immunity to *Streptococcus pneumoniae*. *J Infect Dis*. 154(2):245-56.
88. *Zheng B, Han S, Takahashi Y, Kelsoe G*. 1997. Immunosenescence and germinal center reaction. *Immunol Rev*. 160:63-77.
89. *Tsiagbe VK, Inghirami G, Thorbecke GJ*. 1996. The physiology of germinal centers. *Crit Rev Immunol*. 16(4):381-421.
90. *Eaton SM, Burns EM, Kusser K, Randall TD, Haynes L*. 2004. Age-related defects in CD4 T cell cognate helper function lead to reductions in humoral responses. *J Exp Med*. 200(12):1613-22.
91. *Kovaiou RD, Grubeck-Loebenstien B*. 2006. Age-associated changes within CD4+ T cells. *Immunol Lett*. 107(1):8-14.
92. *Schirmacher V*. 2005. T cell-mediated immunotherapy of metastases: state of the art in 2005. *Expert Opin Biol Ther*. 5(8):1051-68.
93. *Poehlein CH, Ruttinger D, Ma J, Hu HM, Urba WJ, Fox BA*. 2005. Immunotherapy for melanoma: the good, the bad, and the future. *Curr Oncol Rep*. 7(5):383-92.
94. *Kalinski P, Mailliard RB, Giermasz A, Zeh HJ, Basse P, Bartlett DL, Kirkwood JM, Lotze MT, Herberman RB*. 2005. Natural killer-dendritic cell cross-talk in cancer immunotherapy. *Expert Opin Biol Ther*. 5(10):1303-15.

95. *Morgan RA, Dudley ME, Wunderlich JR, Hughes MS, Yang JC, Sherry RM, Royal RE, Topalian SL, Kammula US, Restifo NP, Zheng Z, Nahvi A, de Vries CR, Rogers-Freezer LJ, Mavroukakis SA, Rosenberg SA.* 2006. Cancer regression in patients after transfer of genetically engineered lymphocytes. *Science.* 314(5796):126-9.
96. *Mackensen A, Meidenbauer N, Vogl S, Laumer M, Berger J, Andreesen R.* 2006. Phase I study of adoptive T-cell therapy using antigen-specific CD8+ T cells for the treatment of patients with metastatic melanoma. *J Clin Oncol.* 24(31):5060-9.
97. *Cheadle EJ, Gilham DE, Thistlethwaite FC, Radford JA, Hawkins RE.* 2005. Killing of non-Hodgkin lymphoma cells by autologous CD19 engineered T cells. *Br J Haematol.* 129(3):322-32.
98. *Fishman M, Seigne J.* 2002. Immunotherapy of metastatic renal cell cancer. *Cancer Control.* 9(4):293-304.
99. *Takayama T, Sekine T, Makuuchi M, Yamasaki S, Kosuge T, Yamamoto J, Shimada K, Sakamoto M, Hirohashi S, Ohashi Y, Kakizoe T.* 2000. Adoptive immunotherapy to lower postsurgical recurrence rates of hepatocellular carcinoma: a randomised trial. *Lancet.* 356(9232):802-7.
100. *Jiang J, Xu N, Wu C, Deng H, Lu M, Li M, Xu B, Wu J, Wang R, Xu J, Nilsson-Ehle P.* 2006. Treatment of advanced gastric cancer by chemotherapy combined with autologous cytokine-induced killer cells. *Anticancer Res.* 26(3B):2237-42.
101. *Peethambaram PP, Long HJ.* 2002. Second-line and subsequent therapy for ovarian carcinoma. *Curr Oncol Rep.* 4(2):159-64.
102. *Plautz GE, Miller DW, Barnett GH, Stevens GH, Maffett S, Kim J, Cohen PA, Shu S.* 2000. T cell adoptive immunotherapy of newly diagnosed gliomas. *Clin Cancer Res.* 6(6):2209-18.
103. *To WC, Wood BG, Krauss JC, Strome M, Esclamado RM, Lavertu P, Dasko D, Kim JA, Plautz GE, Leff BE, Smith V, Sandstrom-Wakeling K, Shu S.* 2000. Systemic adoptive T-cell immunotherapy in recurrent and metastatic carcinoma of the head and neck: a phase 1 study. *Arch Otolaryngol Head Neck Surg.* 126(10):1225-31.
104. *Zhou J, Dudley ME, Rosenberg SA, Robbins PF.* 2005. Persistence of multiple tumor-specific T-cell clones is associated with complete tumor regression in a melanoma patient receiving adoptive cell transfer therapy. *J Immunother.* 28(1):53-62.
105. *Mizutani Y, Yoshida O, Miki T.* 1999. Adriamycin-mediated potentiation of cytotoxicity against freshly isolated bladder cancer cells by autologous non-activated peripheral blood lymphocytes and tumor infiltrating lymphocytes. *Urol.* 162(6):2170-5.
106. *No authors listed.* 2006. Sipuleucel-T: APC 8015, APC-8015, prostate cancer vaccine--Dendreon. *Drugs R D.* 7(3):197-201.

107. *Cho HI, Hong YS, Lee MA, Kim EK, Yoon SH, Kim CC, Kim TG.* 2006. Adoptive transfer of Epstein-Barr virus-specific cytotoxic T-lymphocytes for the treatment of angiocentric lymphomas. *Int J Hematol.* 83(1):66-73.

108. *Kashyap A, Snowden J.* 2001. Considerations in the selection of an appropriate conditioning regimen for the treatment of rheumatoid arthritis by autologous peripheral blood stem cell transplantation. *J Rheumatol Suppl.* 64:39-41.

109. *Farge D, Henegar C, Carmagnat M, Daneshpouy M, Marjanovic Z, Rabian C, Ilie D, Douay C, Mounier N, Clave E, Bengoufa D, Cabane J, Marolleau JP, Gluckman E, Charron D, Toubert A.* 2005. Analysis of immune reconstitution after autologous bone marrow transplantation in systemic sclerosis. *Arthritis Rheum.* 52(5):1555-63.

110. *Ings SJ, Balsa C, Leverett D, Mackinnon S, Linch DC, Watts MJ.* 2006. Peripheral blood stem cell yield in 400 normal donors mobilised with granulocyte colony-stimulating factor (G-CSF): impact of age, sex, donor weight and type of G-CSF used. *Br J Haematol.* 134(5):517-25.

December 2006

List of abbreviations

RBC	Red Blood Cells
WBC	White Blood Cells
HSC	Hematopoietic Stem Cell
ABMT	Autologous Bone Marrow Transplantation
DLI	Donor Lymphocyte Infusion
HSCT	Hematopoietic Stem Cell Transplantation
GVL	Graft Versus Leukemia
BMT	Bone Marrow Transplantation
ALL	Acute Lymphoblastic Leukemia
AML	Acute Myeloblastic Leukemia
CLL	Chronic Lymphocytic Leukemia
CMV	Cytomegalovirus
EB	Epstein Barr Virus
DC	Dendritic Cells
NK	Natural Killer
CTL	Cytotoxic T Lymphocyte
TLR	Toll Like Receptor
DM	Dimethylsulfoxyde
AP	Antigen Presenting Cell
CB	Cord Blood (umbilical)
HLA	Human Leucocyte Antigen
AI	Adoptive Immunotherapy