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Cellular therapy in Tuberculosis

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SUMMARY

Cellular therapy now offer promise of potential adjunct therapeutic options for treatment of drug-resistant tuberculosis (TB). We review here the role of Mesenchymal stromal cells, (MSCs), as well as other immune effector cells in the therapy of infectious diseases with a focus on TB. MSCs represent a population of tissue-resident non-hematopoietic adult progenitor cells which home into injured tissues increase the proliferative potential of broncho-alveolar stem cells and restore lung epithelium. MSCs have been shown to be immune-modulatory and anti-inflammatory mediated via cell-cell contacts as well as soluble factors. We discuss the functional profile of MSCs and their potential use for adjunct cellular therapy of multi-drug resistant TB, with the aim of limiting tissue damage, and to convert unproductive inflammatory responses into effective anti-pathogen directed immune responses. Adjunct cellular therapy could potentially offer salvage therapy options for patients with drug-resistant TB, increase clinically relevant anti-*M.tuberculosis* directed immune responses and possibly shorten the duration of anti-TB therapy.

1. Mesenchymal Stromal Cells

Mesenchymal stromal cells (MSCs) represent a population of tissue-resident non-hematopoietic adult progenitor cells, originally identified in the bone marrow,[1] and subsequently in a number of other organs.[2,3] MSCs were identified in the 1970s from cellular suspensions from spleen and bone marrow by their capacity to adhere to plastic – which is still the standard form for culturing MSCs. MSCs are able to form colonies from single cells (explanted *ex vivo*), have fibroblast-like appearance and capacity to differentiate into fat, cartilage and bone. Their function in bone marrow is to facilitate haematopoiesis for expansion of hematopoietic and embryonic stem cells,[4,5] thus may play a role in stimulating cell growth and organization in adult organ tissues.[2] MSCs have been shown to increase the proliferative potential of the so-called bronchoalveolar stem cells[6] and to restore lung epithelium via the transfer of mitochondria to other cells.[7,8] MSCs are defined by CD105, CD90 and CD73 expression and negative for CD45, CD34 and CD14.[9,10] More recent studies show that isolation of MSC from patients with underlying diseases may lead to different phenotypes, mantaining CD105, CD90 and CD73 expression. Bone-marrow derived MSCs may thus represent a mixture of different MSC populations[11] as has also been shown to be true for MSC from lung tissue. Sabatini showed in 2005[3] that a plastic adherent cell population exists in human lungs isolated via BAL[12] or via tissue digestion.[13] These cells are either long-lived or – not mutually exclusive – have the capacity for renewal. Given the fact that MSCs support human stem cells in the bone marrow, as well as bronchoalveolar stem cells, it is most likely that these functions

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may also exist in adult life - and that enrichment of MSCs into damaged lung tissue may aid to re-organize tissue and facilitate healing of chronic, unproductive inflammation associated with *Mcyobacterium tuberculosis (Mtb)* infection.

MSCs are believed to be facilitators of organ homeostasis and tissue repair following infection, neoplasms, damage and 'trauma' in general. MSCs are key cells in connective tissue hierarchy of organs, including the lungs. The roles of MSCs in the lung have recently been extensively reviewed by Sinclair and coworkers.[14] MSCs have been shown to be immune-modulatory, anti-inflammatory and immune-suppressive and most studies have looked at these effects in the allogeneic setting. *In vitro*, MSCs may decrease immune effector functions and aid to expand regulatory T-cells. Both cell-cell contacts as well as soluble factors could be mediating these effects[15,16] particularly on precursor and memory T-cell subpopulations.[16–18] Cell-cell contact appears to be important for expansion of Treg cells, defined by the CD4+CD25high, Foxp+ phenotype.[19,20] The functions of MSCs may be diverse and dictated by the immune-environment; thus making it difficult to predict how MSC will work in patients with lung tissue filled with live *Mtb* bacilli. For instance, co-culture of MSCs with PBMCs leads to prostaglandin E_2 (PGE_2) production in MSCs,[21] PGE_2 and COX2 are increased in the presence of type I interferons and/or TNFα suggesting that the effect of MSCs is influenced by the local cytokine milieu.[15,22]

The production of PGE_2[4,23] from MSCs may be particularly important in balancing unproductive inflammation in TB: High type I interferon levels affect TB disease outcome, increases tissue damage and subsequently increased *Mtb* proliferation. PGE_2 balances the inflammatory cytokines IL-1 and type-I interferons in individuals with latent TB; modulation of this host immune response axis has proven to be effective in preventing death in *Mtb*-infected mice. Studies in mice and analysis of *ex vivo* material from patients with TB demonstrated that IL-1 induces PGE_2 and suppresses type I interferons linked with clinical TB outcome.[24]

Environmental factors (metabolic programming), e.g. oxygen levels, have also been shown to influence the differentiation of MSCs into articular cartilage or epiphyseal cartilage.[25] Of note, more recent studies suggest that MSC may not only differentiate into fat, cartilage or bone, but also into bronchial epithelium, renal epithelium, neuronal tissue as well as cardiomyocytes. This is reflected in a number of studies using MSCs for non-mesenchymal tissues including brain, heart, and kidney diseases.[26] Several studies have now shown that clinical efficacy is not directly related to successful expansion and the level of MSC engraftment, yet to other factors (paracrine), driven by MSCs, which are yet to be identified. One of the aspects of MSCs is the polarization into pro- or anti-inflammatory cells, which appears to be triggered, at least in part, via TLRs. MSCs express TLR3 and TLR4. TLR3-agonists appear to polarize MSC to immune-suppression, whereas TLR4 stimulation leads to immune-stimulation of MSCs.[27] Several components of *Mtb* signal via TLRs and the local effect of MSC - in combination with the cytokine milieu and the TLRs – will contribute to the *Mtb* edited phenotype. One of the factors in *Mtb* infection is lung destruction via fibrosis and collagen synthesis, which is – in part – a TGFβ driven process. We showed that non-human primates that survive longer after *Mtb* challenge have a typical immune phenotype in their lungs, defined by less fibrosis, decreased TGFβ production and increased IL-7 and IL-17 production.[28] Of interest, TGFβ production has been shown to be repressed in TLR3-edited, yet not in TLR4-stimulated MSCs;[27] TLR3-primed MSCs showed up to the 80% reduced TGFβ production, which is mediated via TLR3-induced modulation of TGFβ – downstream effectors SMAD3 and SMAD7; TLR3 versus TLR4 stimulated MSCs also show differential IDO and PGE_2 production, which also underlines the local immune-editing milieu of *Mtb* infected tissues. PGE_2 converts macrophages into an IL-10 – producing phenotype. Immunomodulatory properties include the production of IL-1 receptor antagonists and the TSG-6 protein (anti-inflammatory protein TNFα stimulated gene protein 6).[29]

A number of clinical trials using MSCs as immune-modulatory agents or as stimulators for tissue generation have been reported. MSCs are being used for corticosteroid-resistant Graft versus Host Disease (GVHD) (*i.e.* inflammatory reactions after hematopoietic stem cell transplantation), and for treatment of other autoimmune diseases (Multiple Sclerosis, Crohn's disease etc.).[30,31] Sinclair and colleagues[14] in their phase I clinical study of MSC infusions in an allogeneic setting established safety of the allogeneic MSC infusion, with 2×10^6 cells / kg i.v. twice weekly for two weeks. The aim was to offer MSC for treatment of complications after lung transplantation as well as for the treatment of idiopathic lung fibrosis (www.clinicaltrial.gov). Another study evaluated the intra-tracheal administration of umbilical cord derived MSCs in children with bronchopulmonary dysplasia (www.clinicaltrials.gov/ct2/show/NCT01297205).

2. MSCs and infection

MSCs are susceptible to infection by several intracelullar pathogens such as *Mtb*, Influenza virus[30] and Herpesvirus-6 infection.[32] Conversely, MSCs have been shown to improve survival[33] in bacterial infections of mice which supports the concept as stated above that organ-damaging cascades in infections can be curbed with MSC treatment:[34] MSCs reduce inflammation–associated lung damage.[35,36] The safety of MSC therapy has recently been extensively reviewed by Lalu and coworkers.[37] Other beneficial effects may be the production of exosomes and microvesicles from MSCs which has been studied in the interaction of MSC and cancer cells,[38] but not in the context of MSC and pathogens. This is also a potential new area of investigation: if the signalling proteins and miRNA in the exosomes and microvesicles can be identified, potentially the cell therapy infusions can be obviated to the far simpler protein/miRNA infusions – if exosomal delivery of signals and proteins turns out to be biologically and clinically relevant in infections. Nauta and Fibbe reviewed the immunomodulatory properties of MSCs and showed the impact of MSCs on T-cell functions, including cytotoxicity; on dendritic cell functions (impaired CD83 and HLA-DR expression); B-cell function and NK-cells, defined by proliferation and cytotoxicity.[39] The type and severity of adverse effects may differ based on patient populations and the underlying disease, as well as the MSC characteristics used for expansion and subsequent therapy. A meta-analysis of the randomised clinical trials examining autologous and allogeneic MSC therapy in patients, searching MEDLINE, EMBASE, and the Cochrane Central Register of Controlled Trials (till June 2011) did not detect associations between infusion toxicity, organ systemic complications, infections, death or malignancies. Whilst an association was identified with MSCs and transient fever, the application of MSC was found to be safe in 36 clinical studies. In addition, we have shown that MSC application in patients with MDR and XDR TB is safe.[40]

A recent publication has addressed the increasing use of MSC in the treatment of acute and chronic graft versus host disease (GVHD) in transplant patients with immunomodulatory effects and have suggested more prospective randomized controlled trials for optimisation of the MSC therapy.[31] Another recent report from the NIH clinical centre using third-party early passage (up to passage 3) MSCs infused at 2×10^6 MSCs/kg body weight IV weekly for 3 doses in a phase I clinical trial for patients with steroid-refractory GVHD following post-transplant complications established safety as well as significant rapid clinical responses and biomarker normalisation among the majority of the study participants. The study observed positive outcomes in patients with a relatively intact immune system with higher absolute lymphocyte counts and favourable cytokine and T cell phenotype patterns.[41]

A recent review has highlighted the promise of MSC for Acute Respiratory Distress syndrome and sepsis while elucidating the challenges and bottlenecks in the field and the clinical development.[42] The review cited the successful small randomised trial of adipose tissue derived MSC in 12 ARDS patients in China and also of the two ongoing allogeneic bone-marrow derived MSC trials in US as well as an upcoming trial in Canada for patients with Septic Shock.

3. Principle of adjunct MSC treatment for TB

MSCs dampen inflammation through an array of interactions with innate and adaptive immune cells thereby modulating immune responses. MSCs, which constitute ~0.001% of bone marrow mononuclear cells (proportion declines over age), can be easily expanded *ex vivo* in culture and when re-infused in patients they home to sites of injury and inflammation promoting tissue repair. The culture conditions, degree of expansion and the final MSCs preparations, may vary influencing clinical outcome.

Enhanced *Mtb*-antigen specific responses were observed following MSC infusion in a Phase I study conducted in Belarus patients with MDR-TB.[40]

An ongoing study of adjuvant autologous MSC therapy in South African patients with MDR/XDR-TB is establishing the safety in patients with MDR/XDR TB in Durban, King Dinuzulu Hospital Complex and is investigating immunological mechanisms of anti-TB responses and markers of a response to therapy. Specific efforts have been made to study responses to MSC treatment defined by HR-CT imaging as well as to assess the best incremental value of this adjuvant therapy in the subset of patients who would benefit from this mode of cellular therapy, compared to other possible immune-interventions targeting the host immune response.[43]

4. MSC clinical trials registered at www.Clinicaltrials.gov/

On searching the www.clinicaltrials.gov web site, accessed on November 15, 2014 using the term "mesenchymal stem cell", 437 studies were identified. Figure 1 depicts the clinical trials/studies by geographic location with a majority of studies ongoing in China, Europe and USA. Using the search term "mesenchymal stromal cell", 60 studies were found across the globe (Figure 1) with 32 open ongoing studies and the highest number (8) in the category of vascular diseases with a focus on Ischemic Stroke; 6 studies on Central Nervous System Diseases ranging from Ischemic Stroke, Amyotrophic Lateral Sclerosis, Parkinson's Disease, Spinal cord injury, 6 studies in Musculoskeletal diseases – mostly in osteoarthritis; 6 studies in Digestive system diseases in Crohn's disease; 4 studies in Graft vs Host Disease (GvHD, 2 at Karolinska Institutet) and 3 studies in autoimmune diseases, i.e in Multiple Sclerosis. Note that the safety evaluation of these trials did not show any adverse effects nor an increased risk to viral or bacterial infections. There are no ongoing studies with MSC therapy in Africa and the ongoing Phase Ib/IIa clinical trial of the use of autologous bone-marrow-derived MSCs as adjunct treatment for MDR/XDR-TB is the first to be conducted in Durban, South Africa.

5. Perspectives

Cellular therapy is today attracting attention and provides hope of an alternative adjunct treatment for unmet clinical needs for a range of chronic disorders. The concept from the large-scale pharma-driven industrial production of a drug or biopharmaceutical, may shift for certain clinical indications to a more "personalized", precision medicine concept. This will require coordination and harmonisation of efforts various stakeholders to meet international GMP and GCP standards and to show the added value of MSCs as adjunct- or salvage therapy for a range of chronic infectious diseases, including Tuberculosis.

6. The evolution of cellular therapy and the lessons from cancer treatment

Adoptive Cell Therapy (ACT) has been used in the field of metastatic cancer, and it involves isolation of antigen-specific immune cells, their expansion and activation *ex vivo* followed by

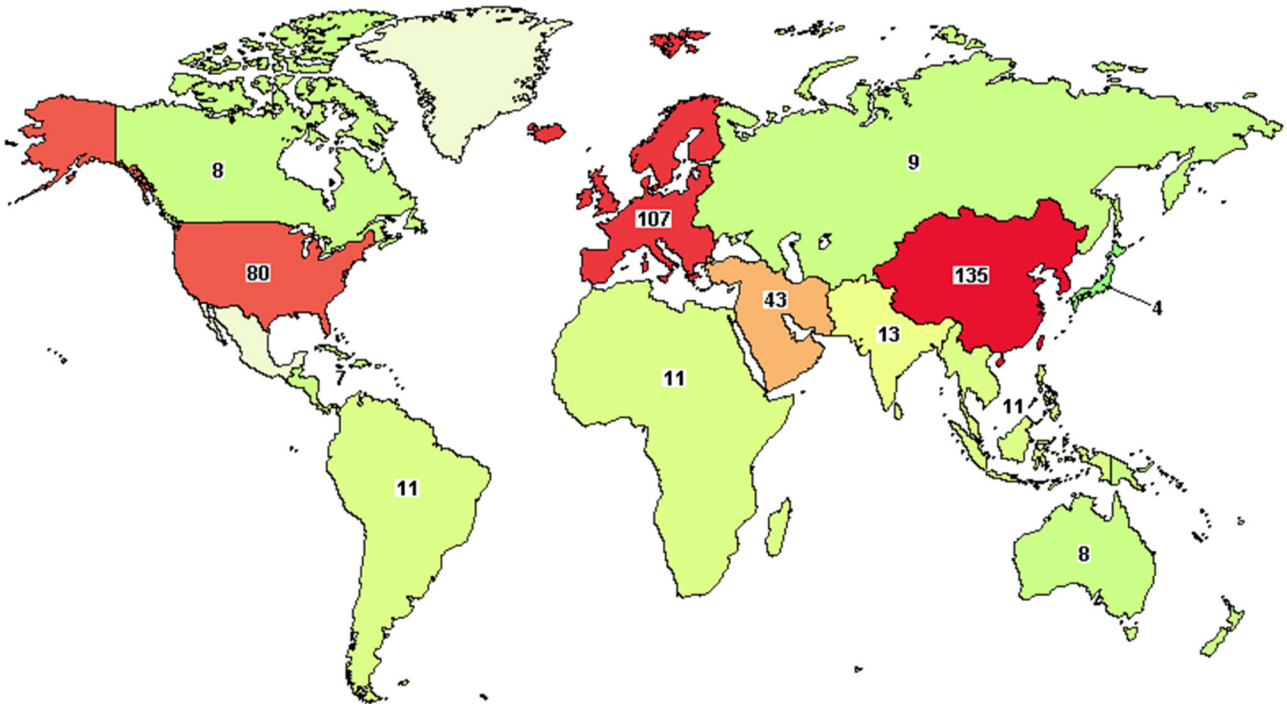

Figure 1. Clinical studies with MSC across different geographical locations.

subsequent re-infusion to the autologous host. The 'lessons learned' in the context of anti-cancer directed therapies may be of benefit to the use of 'cellular' therapies for the adjunct treatment of TB. An overview of cellular treatments in the fields of cancer and infectious diseases is provided in Table 1.

Cellular therapy in cancer settings started with lymphokine activated killer (LAK) cells given intravenously in combination with recombinant interleukin-2 (rIL-2), generated from autologous lymphocytes harvested from patients by leukapheresis, followed by activating the cells with rIL-2.[44] Objective tumor reduction could be achieved in patients with metastatic renal cell carcinoma, melanoma and colorectal carcinoma. Toxicities of high-dose IL-2, e.g. fluid retention and pulmonary edema limited this therapy. This approach was further optimised with the addition of other biological response modifiers (such as interferons), and chemotherapeutic agents (especially cyclophosphamide, acting via removal of regulatory T-cells, Tregs) aiming to induce long-term memory T-cell responses directed against MHC class I- or MHC class II-presented epitopes to target-specific T-cells.[45]

Tumor infiltrating lymphocytes (TILs) were isolated from freshly resected melanomas and expanded *ex vivo* before infusing autologous TILs back to the patient along with rIL-2 based on the idea that TIL are enriched for antigen-experienced T-cells that would benefit from ex vivo expansion and removal of adverse factors, such as TGFβ or IL-10, elaborated in the tumor microenvironment. 'Conditioning', i.e. treatment – induced lymphophenia, that provides 'space' for expansion of the adoptively transferred antigen-specific T-cells, reduces the competition for growth factors leads to the removal of Tregs associated with increased objective clinical responses.[46]

Lymphocytes were modified by retroviral gene transduction with a neomycin marker[47] in order to track the infused immune cells in the patients. These data provided evidence that objective clinical responses are generated by (infused) T-cells infiltrating into tumor lesions, it also provided clinically relevant information on how retrovirus-mediated gene transfer in humans can be administered safely and effectively.

To strengthen the T cell reactivity to nominal target antigen(s), and to overcome T-cell tolerance, T cells are engineered through regulated introduction of genes that encode high affinity tumor-targeting T cell receptor (TCRs) or synthetic Chimeric Antigen Receptors (CARs). CARs use an engineered antibody fragment to recognise the target cell and link this artificially to a number of signalling domain proteins within the T cell designed to "switch the T cell on" once the antibody recognition fragment (for example, the CD19 B cell protein) has bound to a target cell. However, although not HLA-restricted, CARs are limited by the low number of antibody targets available to re-direct the T cell.[48,49] Anti-pathogen directed CARs may also represent a viable option for the treatment of infectious pathogens, although caution must be exercised concerning dangerous 'off-target toxicity' that may cause serious medical complications, some of them associated with the 'cytokine storm' induced by high antigen load.

Transformed or virally infected cells typically present processed peptides (epitopes) from viral proteins, this is also true for tumor-associated (mutant or non-mutant) target epitopes on their surface in association with major-histocompatibility antigens (MHC) class I or – class II. T cells are educated early in the development process in thymus to prevent recognition of self-antigen(s) reflected in the very low affinity of binding to self-antigen(s). Several groups have now established novel technology to enhance the natural TCR affinity to either viral or cancer protein epitopes overcoming these obstacles to develop TCRs that could be used to target (cancer or infectious pathogen) specific proteins displayed by MHC class I or – class II molecules; however, the TCR would have to be transferred into recipient effector T-cells, using either lentiviral vectors, non-viral plasmid-based vector systems, or alternatively, RNA-based transfer of TCRs conferring immune-reactivity, i.e. reactivity to molecularly defined targets displayed by transformed or infected cells. Further refinement in the T-cell engineering was achieved through combinatorial antigen recognition with balanced signalling by transducing T cells with both a CAR providing suboptimal activation upon binding of one antigen and a chimeric costimulatory receptor (CCR) that recognises a second antigen, thereby promoting selective target eradication.[50]

7. TIL & DC combination

Induction of therapeutically useful antitumor immunity in cancer patients requires the development of powerful vaccination protocols due to the preexisting antigenic load and immunosuppressive environment within a tumor. Autologous Dendritic cells (DC) loaded *ex vivo* with tumor antigens by transfecting whole RNA of the resected tumor have been tried successfully in metastatic melanoma patients,[51,52] an approach that could also be discussed in chronic viral or bacterial infections.

TIL infusion has therefore been combined with dendritic cell (DC) vaccination[52] (for patients with stage IV melanoma) to induce strong and long-lived T-cell memory responses. Analysis of the T cell receptor repertoire revealed the presence of highly dominant clones in most infusion products, and many of these could be detected in the circulation for weeks after T cell transfer. It is

Table 1
Summary of cellular treatments used for cancer and infectious diseases.

Cell type	Adjuvant/ Biological Response Modifier used	Clinical conditions used in	Ref
LAK cells	rIL-2	Metastatic renal cell carcinoma, Melanoma & Colorectal carcinoma	44
TIL	IL-2 & focus with Private Ag	Melanoma	46
		Epithelial Cancer	61
	Anti-CTLA4	Metastatic Melanoma	62
	Anti-CTLA4, Anti-PD-L1, Anti-4-1-BB, Anti-CD40	Advanced cancer	63
DC	Transfected by t-RNA from Resected tumor	Metastatic Melanoma	8
DC + TIL		Stage IV Melanoma	9
CMV-specific CD8 T cell clones	IL-2 + repeated Ag	Post-transplant CMV inf	10,11
CD19 CAR T Cells		ALL, CLL, NHL	17
CAR-modified T cells		HIV	64–66
Anti-viral reactive T cells		Post-Transplant patients against CMV/ENV/HP6/Adeno	12,67
NK Cells		Advanced non-small cell lung cancer	68

speculated that the administration of lymphodepleting chemotherapy and IL-2 will most likely increase treatment efficacy with this approach.

In an infectious disease context, initial cell therapy was tried in life-threatening infections such as cytomegalovirus (CMV) infections in post-transplant settings with allogeneic bone marrow transplant as a consequence of severe and prolonged immunodeficiency. CMV specific CD8+ T cell clones were generated from peripheral blood mononuclear cells of the respective bone marrow donors using repeated antigen stimulation along with IL-2 and were adoptively transferred to the post-transplant patients. These CMV-specific CD8+ T cells persisted for at least 12 weeks and were effectively *in vivo* augmented by transfer of CMV specific CD4+ T cells.[53,54]

T cells targeting a range of viral antigens derived from EBV, CMV, and AdV were reproducibly generated in a single culture over a 2-3-week period, using methods that exclude all viral components and employ a much-simplified culture technology. When administered to recipients of post-transplant patients with active CMV (n = 3), AdV (n = 1), EBV (n = 2), EBV+AdV (n = 2) or CMV+AdV (n = 2) infections, the cells produced complete virological responses in 80%, including all patients with dual infections correlating with an increase in the frequency of T cells directed against the infecting pathogens without immediate or delayed toxicities.[55]

''Off the shelf,'' or banked, partially human leukocyte antigen (HLA)–matched multi-virus-specific T cells (mVSTs) were generated using single T cell lines from stem cell donors upon restimulations with overlapping peptide libraries for up to five viruses (AdV, EBV, CMV, BKV, and HHV6) representing the most frequent causes of viral morbidity and mortality after HSCT. This was tried in 11 recipients of allogeneic transplants, 8 of whom had up to four active infections with the targeted viruses and was proven safe in all subjects and produced an overall 94% virological and clinical response rate that was sustained long-term.[56] TB along with Epstein-Barr virus-associated lymphoproliferative disorders and cytomegalovirus infection has been reported in an allogeneic stem cell transplant recipient for refractory acute myeloid leukemia, uncommon in a TB non-endemic region.[57]

8. IMMUNOLOGICAL CHECKPOINT INHIBITORS: Blocking CTLA-4 and PD-1

Many attempts are ongoing to integrate further controls in CAR based ACT (Adoptive Cell Therapy) by regulating gene expression and the immune cell kinetics *in vivo* in combination with novel checkpoint inhibitors and cytokines. Our understanding concerning the role of cell-surface inhibitory molecules has increased; blocking these inhibitory receptors, termed 'checkpoint modulators', such as anti-CTLA- 4 (cytotoxic T-lymphocyte-associated protein 4; also known as CD152 which act as a major negative regulator of T-cell responses) in patients with metastatic melanoma leads to increased overall survival[55] in a subset of patients.

The Programmed death 1 receptor (PD-1) and its ligands (PD-Ls) molecules inhibit T cell effector functions during active infection. Furthermore, the simultaneous blockage of the inhibitory receptor PD-1 together with the activation of the costimulatory protein signaling lymphocytic activation molecule resulted in promotion of protective IFN-γ responses to *Mtb*, even in patients with weak cell-mediated immunity against the pathogen. PD-1 has been demonstrated to interfere with T cell effector functions against *Mtb*, suggesting its key regulatory role during the immune response of the host to the pathogen.[58] Anti-PD-1 responses will most likely help to overcome T-cell anergy in patients with TB, although caution has to be exercised: i) treating patients with reagents targeting PD-1 may lead to autoimmune responses, ii) PD-1 expression on immune cells may not only indicate immune 'exhaustion', but also identify T-cells that are antigen-experienced (and therefore interfere with pathogen-directed T cells).

Upon infection, antigen-specific CD4 and CD8 T cells undergo activation and perform effector functions. In chronic infections such as TB, prolonged persistence of the *Mtb* leads to alteration of function of pathogen-specific T cells, ultimately resulting in immune exhaustion. CD4 T cells have been shown to be exhausted and functionally unresponsive following persistent CMV infections and this has also been seen in CD8+ T cells in metastasis from melanoma patients. TLRs (Toll like receptors) operate synergistically to induce optimal immune responses against intracellular pathogens. In an TB animal model, prolonged TCR stimulation of naive CD4 T cells under Th1-polarizing conditions resulted in an exhausted phenotype which could be limited through TLR-2 by downregulating the expression of PD-1 and Lag-3 and increasing the expression of IFNγ, Bcl-2, and IL-2 through Tbet dependent signalling.[59] PD-1– and Lag-3–blocking therapy may therefore hold promise in treating chronic infections.

In summary, antibodies targeting negative regulatory molecules such as programmed death 1 (PD-1) and cytotoxic T-cell lymphocyte-associated antigen 4 (CTLA-4) can be infused to release the brakes on natural T cells response to transformed cells, thereby augmenting the response. Chemotherapy can reduce immune suppressive cells such as Tregs and myeloid-derived suppressor cells (MDSC) in addition to its direct effect on the tumor cells. Adoptive T-cell transfer strategies using clonally expanded cytotoxic T cells or T cells engineered to express TCRs or CARs are being tested in various cancers including haematological malignancies;[60] TCRs or CARS directed against M. tuberculosis may have the potential in the adjunct treatment of tuberculosis.

9. Outlook

Tran and colleagues have merged T cell therapy with tumor exome sequencing and provided a proof of concept that T cells recognize tumor-specific mutations; culturing and expanding these T cells followed by subsequent transfer of these T-cells back to the patient has been proven to be sufficient to mediate tumor regression. Resected lung metastases from a patient with metastatic cholangiocarcinoma were used as a source of tumor and T cells. After identification of 26 nonsynonymous mutations, a CD4+ T cell population that specifically recognized a mutant epitope from erbb2-interacting protein (ERBB2IP) was identified, expanded, and cloned; the T cell receptor was sequenced; and its specificity confirmed.[61] The patient received two infusions of cultured and expanded tumor-infiltrating lymphocytes; conditioning chemotherapy and cytokine support were administered to improve engraftment of the cells. The patient experienced a marked tumor regression after each infusion: This is a prime example that the patient's immune system targets mutations and that T-cells directed against mutant epitopes are able to confer tumor regression. A similar situation may be feasible for T-cells recognizing wild-type and/or mutant epitopes in *Mtb*.

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