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44

Stem-Like Characteristics in a Subset of CD161 Expressing Human Memory CD4+ T Cells Facilitates Their Survival After Chemotherapy

Abdullah Alsuliman [1], Ahmad Khoder [1], Kate Stringaris [1], Takuya Sekine [1], Bonnie Razzaghi [1], Hugues de Lavallade [1], Anushruthi Sarvaria [1], David Marin [1], Katayoun Rezvani [1,2]. [1] Haematology, Imperial College, London, United Kingdom; [2] Stem Cell Transplantation & Cellular Therapy, MD Anderson Cancer Center, Houston, TX

$CD4^+$ helper T cells are indispensible in shaping the adaptive immune system and play an important role in protection against infections. The establishment of a wide range of long-lived pathogen-specific T cells, which are ready-to-act upon second encounter with the specific pathogen, is a fundamental property of the adaptive immune response. However, the mechanisms by which antigen specific helper T are sustained long-term and resist insult by lymphocytotoxic agents is not well defined. A recent report described the existence of a long-lived $CD8^+$ memory T cell population with stem cell-like properties (Turtle et al., Immunity 2009) including the ability to efflux cellular toxins through the ATP-binding cassette (ABC)—superfamily multidrug efflux protein ABCB1. We hypothesized that a subset of memory T cells with stem-like properties also exists within the $CD4^+$ T cell compartment.

Here we identified a subset of memory $CD4^+$ T cells with rapid drug-effluxing ability. We showed that drug effluxing $CD4^+$T cells have a remarkable phenotypic similarity to $CD8^+$ stem-like memory T cells described by Turtle et al and are defined as $CD161^+CD95^+CD45RA^-CD127^{hi}CD28^+CD25^{int}$. Furthermore, effluxing $CD4^+CD161^+$ T cells proliferated poorly in response to anti-CD3/CD28 stimulation compared to their $CD161^-$ counterpart, indicating that this subset of quiescent T cells may resist insult by cytotoxic agents through a number of different mechanisms.

We demonstrated that $CD4^+CD161^+$ T cells are enriched within the viral-specific T cell repertoire and persist after chemotherapy for AML. The high ABCB1-mediated drug efflux capacity of $CD4^+$ $CD161^+$ memory cells facilitated their resistance to daunorubicin in vitro and, this resistance was abrogated by the addition of competitive inhibitors of ABCB1 and ABCC1. Finally we demonstrated that following vaccination with seasonal influenza vaccine (Flu), CD161 expression was significantly higher on Flu-specific CD4+ T cells at 2 years, compared to 4 weeks post-vaccination, ($74.3\% \pm 4.6\%$ vs. $49.3\% \pm 3.5\%$ $P \leq .007$), suggesting that CD161 is a marker for long-lived Ag specific memory T cells. These data have significant implications for the development of novel adoptive therapy strategies using long-lived stem-like memory T cells.

45

Autologous aGVHD Associated with Infusion of T-Cells with Engineered Specificity for NY-ESO-1 and LAGE-1 Following High-Dose Melphalan and ASCT in Patients with Multiple Myeloma

Alfred L. Garfall [1], Michael Kalos [2], Emma E. Furth [3], Dan Vogl [1], Brendan Weiss [1], Joshua Cantor [3], Minnal Gupta [3], Bruce Levine [2], Aaron Rapoport [4], Lilliam Ribeiro [5], Bent Jakobsen [5], Dominic Smethurst [5], Gwendolyn Binder-Scholl [5], Carl H. June [2], Edward A. Stadtmauer [1]. [1] Division of Hematology & Oncology, Department of Medicine, Abramson Cancer Center and Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA; [2] Department of Pathology & Laboratory Medicine, Abramson Cancer Center and Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA; [3] Department of Pathology & Laboratory Medicine, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA; [4] Greenebaum Cancer Center, University of Maryland, Baltimore, MD; [5] Adaptimmune, LLC, Philadelphia, PA

Autologous stem cell transplantation (auto-SCT) is uncommonly associated with acute graft-versus-host disease (aGVHD)-like syndromes that are typically mild and self-limited; incidence is increased when auto-SCT is coupled with infusion of *ex vivo* expanded/costimulated T-cells. We describe here a gastrointestinal GVHD-like syndrome in multiple myeloma (MM) patients treated with auto-SCT and T-regulatory-cell (T_{reg})-depleted, autologous T-cells genetically modified to express a T-cell receptor with engineered specificity for a HLA-A*02-restricted epitope common to cancer-testis antigens NY-ESO-1 and LAGE-1. Subjects were $HLA\text{-}A2^+$ and had high-risk MM with confirmed target expression. After initial therapy, T-cells were harvested via apheresis for transduction and culture with anti-CD3/-CD28-antibody-conjugated microbeads. Stem cells were then mobilized with cyclophosphamide +/- bortezomib. Subjects received melphalan 200 mg/m^2 on day -2 and $>2 \times 10^6$ $CD34^+$ cells/kg on day 0. On day +2, autologous T-cells were infused (avg. $9x10^9$, range $7\text{-}10x10^9$; gene-modified avg. 33%, range 18-38%). Of the first 3 subjects treated, all developed persistent fever and grade 3-4 diarrhea beginning between days 2-7. Abdominal imaging was obtained in 2 subjects and demonstrated marked wall thickening in the small and large bowel. Bowel biopsies from all subjects demonstrated aGVHD. The syndrome resolved spontaneously on day +37 in subject #1, but subjects #2 and #3 required immunosuppression with steroids. With recognition of this syndrome, prophylactic administration of oral budesonide and beclomethasone was implemented. The syndrome was then observed in only 1 of 7 subsequently treated subjects; in this subject, symptoms developed only after prophylactic budesonide was discontinued due to inability to administer the drug while the subject was ventilator-dependent with pneumonia. In subjects #2 and #3, bowel biopsy specimens were assayed by qPCR for the presence of the engineered cells, which were found to be present in all biopsy specimens from both subjects. Q-RT-PCR analysis of biopsy tissues showed absence of transcripts for both NY-ESO-1 and LAGE-1 antigens and accumulation. In conclusion, immunotherapy with T_{reg}-depleted, NY-ESO-1/LAGE-directed autologous T-cells after high-dose melphalan is associated with a steroid-responsive and preventable immune enteritis possibly mediated by infiltration of the GI tract by the engineered autologous T-cells.

46

Epitopes of CMVpp65 Co-Presented by Multiple Allelic Variants of HLA Class-I Antigens: Implications for Adoptive Immunotherapy for CMV Using Third Party Donor – Derived CMV Specific CTLs

Aisha Nasreen Hasan [1], Guenther Koehne [2], Annamalai Selvakumar [3], Ekaterina S. Doubrovina [4], Susan Prockop [5], Richard O'Reilly [5]. [1] Department of Pediatrics, Pediatric Bone Marrow Transplantation Service, Memorial Sloan-Kettering Cancer Center, New York, NY; [2] Department of Medicine, Adult Bone Marrow Transplant Service, Memorial Sloan-Kettering Cancer Center, New York, NY; [3] Sloan Kettering