3437 Development of a proliferation assay for immune humanized mouse lymphocytes

M. K. Chow, E. A. Svyatova, and *K. E. Howard*. U.S Food & Drug Administration, Silver Spring, MD.

Background and Purpose: Immune-humanized mice could be an important tool for assessing immunogenicity of human biological drug products, but functional assays are needed to assess T cells *ex vivo*, following *in vivo* dosing. In this study, we developed and optimized a proliferation assay using immune-humanized mouse T cells. Functional assays of T cells are important tools to assess how well they respond to *ex vivo* stimulation. Proliferation assays have been widely used to detect cell division in culture in response to different antigens. Cell division is measured using dyes such as carboxyfluorescein succinimidyl ester (CFSE) by flow cytometry at the end of culture with greater dilution of the dye indicating more cell division has occurred. This allows for a better understanding of the immunologic response of T cells. Using literature for proliferation assays developed for murine cells as a starting point, we evaluated different parameters including cell concentration and antigen concentration of concanavalin A (ConA) and infliximab, to optimize the assay using immune-humanized mouse splenocytes and lymph node cells. **Methods:** Splenocytes and lymph node cells were isolated from either bone marrow-liver-thymus (BLT) or CD34 humanized mice. They were used either fresh or after thawing from cryopreservation. Cells were counted and stained with a CellTrace™ Blue (Sigma) dye at a concentration of 5 µM with staining procedures as recommended by manufacturers protocol. We initially tested cell concentrations ranging from 1.0 x 10^5 to 3.0 x 10^5 per well in 500ul of serum-based media in a 48-well tissue culture plate with 20, 30, or 40 ug/mL of ConA, a positive control, for 66-72 hours of culture at 37ºC and 5% CO2. After determining an optimal concentration of cells and positive control, we tested cells from mice that had been treated *in vivo* with infliximab at concentrations of 10, 25, or 50 ug/mL to determine the optimal concentrations for *ex vivo* stimulation. Stained and unstained cells in culture media were used as negative controls. Following culture, cells were washed with 2mM EDTA wash buffer, placed in a 96-well plate, and collected on a Cytek Aurora flow cytometer. Data was analyzed using FCS Express software. **Results:** Initially, we evaluated different cell concentrations, ranging from 1.0 x 10^5 to 3.0 x 10^5 per well. We determined the optimal concentration was 2.0 x 10^5. Cell concentrations less than 2.0 x 10^5 resulted in less-than-optimal numbers to analyze at the end of the assay, and cell concentrations greater than 2.0 x 10^5, produced more cells than were needed, and used up valuable sample that could be employed in other assays. The positive control was ConA, and we found that concentrations greater than 20 ug/mL induced excessive stimulation and caused cells to die during culture period, leaving little sample to analyze. For infliximab, concentrations greater than 10 ug/mL also induced excessive stimulation that caused cells to not be viable for analysis at the end of the culture period. **Conclusions:** We successfully developed a proliferation assay that can be used with immune-humanized models. This assay allows for functional analysis of proliferative capacity to specific antigens, without consuming an excessive number of cells and will enable *ex vivo* assessment of immunogenicity of biological drug products.

3439 High-dose IL-2 reduces IL-2R signaling capacity in T cells

C. Sommer[1,2], S. Jacob[1,2], S. Dehmel[1,2], V. Neuhaus[1,2], A. Braun[1,2], and K. Sewald[1,2]. [1]Fraunhofer Institute for Toxicology and Experimental Medicine, Hannover, Germany; and [2]Member of the German Center for Lung Research (DZL), Biomedical Research in Endstage and Obstructive Lung Disease Hannover (BREATH) research network, Hannover, Germany. Sponsor: *A. Bitsch*

Background and Purpose: Although high interleukin-2 (IL-2) doses were a promising anti-cancer therapy, systemic toxicity on organs such as skin, liver, or vasculature limit the use of the therapeutic. Still, modified IL-2 molecules are extensively being studied for tumor therapy and treatment of inflammatory diseases. To prevent IL-2-induced immunotoxicities in future IL-2-based therapies, a better understanding of molecular and cellular mechanisms is needed. Similar autoimmune-like organ manifestations such as dermatitis are observed in patients with mutations in the IL-2 receptor β subunit (IL2Rβ). In these patients, T cells show reduced IL2R signaling while signaling in natural killer (NK) cells is relatively unaffected. IL-2Rβ, together with the common gamma chain (IL-2Rγ; e.g. shared by IL-7), forms the intermediate-affinity IL-2Rβγ, shared by IL-15 and expressed on resting conventional T and NK cells. Additional expression of IL-2Rα forms the high-affinity trimeric IL-2Rαβγ, specific for IL-2 and abundantly expressed on regulatory T cells (Tregs). Upon IL-2 binding, IL-2Rβ and IL-2Rγ are intracellularly degraded, while IL-2Rα is recycled to the cell surface. Based on the differential regulation of IL-2R subunits and observations in patients with *IL2RB* mutations, we hypothesize that high-dose IL-2 (hdIL-2) stimulation decreases IL-2Rβ surface expression and in turn reduces IL-2R signaling capacity especially in T cells but not NK cells. **Methods:** Human peripheral blood mononuclear cells (PBMCs) were continuously stimulated with 1-10,000 IU/mL IL-2 (aldesleukin) for up to 7 days. Receptor surface expression and IL2R signaling (phosphorylation of STAT5) of cell subsets were measured using flow cytometry. To investigate the impact of continuous IL-2 stimulation on IL-2R signaling capacity, PBMCs stimulated with increasing IL-2 concentrations for 7 days were washed, rested in medium without IL-2 for 1 h, and re-stimulated with hdIL-2, IL-15, or IL-7 for 15 min before analysis of pSTAT5. **Results:** While IL2Rβ surface expression remained high on CD8+ T cells and NK cells after 15 min of hdIL-2 stimulation, significant decreases on CD4+ T cell subsets to levels below 0.4% were observed (vs. 2.5% for unstimulated T helper cells (Th) and 26.1% for regulatory T cells (Tregs)). Prolonged IL-2 exposure (up to 7 days) reduced IL-2Rβ expression on CD8+ T cells to 0.2%. Expression on NK cells also was reduced but 55.8% of cells remained IL 2Rβ+. In comparison, IL-2Rα expression was significantly increased on hdIL-2-stimulated T cell subsets and NK cells (2.1-2.6-fold increase in mean fluorescence intensity (MFI) compared to unstimulated control) after 7 days of IL-2 stimulation. IL-2Rγ expression was slightly but statistically not significantly decreased on CD4+ T cells (1.5-fold and 2.1-fold decrease in MFI on CD4+ Th and Tregs, respectively) and remained unaltered on CD8+ T and NK cells. IL-2 re-stimulation of CD4+ and CD8+ T cells stimulated with hdIL-2 for 7 days showed lower pSTAT5 signal than unstimulated cells (57.1% vs. 80.2% pSTAT5+ for CD4+ Th, 57.4% vs. 94.1% for Tregs, 67.3% vs. 90.3% for CD8+ T cells, respectively). In contrast, pSTAT5 signal in hdIL-2-stimulated NK cells remained stable around 80% upon re-stimulation with IL2. Similarly, IL-15 re-stimulation of T cells which previously have been stimulated with hdIL2 significantly reduced pSTAT5 compared to cells without prior IL-2 stimulation, while pSTAT5 in NK cells was unaltered. IL-7 re-stimulation of hdIL-2-stimulated CD8+ T cells induced slightly decreased pSTAT5 compared to cells without prior IL-2 stimulation (85.9% vs. 94.3% pSTAT5) and these decreases were more prominent, but still not statistically significant in hdIL-2-stimulated CD4+ T cells (69.0% vs. 97.7% pSTAT5+ CD4+ Th, 38.1% vs. 60.3% pSTAT5+ Tregs). IL-7 re-stimulation of NK cells after hdIL-2 stimulation increased pSTAT5 to 68.7% compared to cells without prior IL-2 stimulation (28.9% pSTAT5+). **Conclusions:** While NK cells retained relatively high IL-2Rβ expression, IL-2Rβ was basically absent on T cells after continuous hdIL-2 stimulation. These cell-specific differences were mirrored in reduced IL-2R signaling capacity which only was observed in T but not in NK cells. Overall, CD4+ T cells seem to be more broadly affected by hdIL-2 treatment as evidenced by immediate decreases of IL-2Rβ surface expression, slight decreases of IL-2Ry surface expression after continuous IL-2 exposure, and concomitant decreases in IL-7 signaling. This might have implications for cell function, especially of CD4+ T cells and could therefore induce autoimmune-like side effects such as skin rashes during IL-2 therapy, similar to patients suffering from *IL2RB* mutations.

3441 Evaluation of Aerosolized 3D Printer Emissions in a Murine Asthma Model

L. G. Jackson, L. M. Weatherly, *W. K. Mandler*, W. McKinney, M. P. Cooper, *Y. Qian*, and *S. E. Anderson*. NIOSH, Morgantown, WV.

Background and Purpose: Occupational asthma is a serious public health burden caused by exposure to various agents in the workplace. Fused filament fabrication 3D printing is a new and emerging industry that uses a variety of filament materials capable of producing ultrafine particulates and volatile organic compounds. Preliminary studies suggest an association between exposure and increased incidence of asthma. However, this relationship has not been thoroughly investigated. The goal of this study was to investigate pulmonary function in mice exposed to inhaled emissions from 3D printing with a polycarbonate filament using a mouse model of asthma. **Methods:** Female Balb/c mice were exposed to aerosolized emissions generated from 3D printing at 2.5 mg/m^3, for 30 days, 4 h/day, 4 days/week or air control. During exposures, mice were sensitized to the experimental allergen ovalbumin (OVA) by intraperitoneal injections (5 µg OVA, 2 mg Alum in 200 µl PBS) on days 1 and 8 of inhalation exposures, and by intratracheal instillations on days 19 and 31 (60 µg OVA in 60 µl PBS). To evaluate the effects of 3D printing emission on pulmonary function, whole-body plethysmography (PenH) was used to measure bronchial reactivity 24 hours after the last exposure. Twenty-four hours after the OVA challenge, the animals were euthanized and tissues including BALF, lung, and blood were collected for analysis. **Results:** An increase in airway hyperreactivity was observed in the mice exposed to the 3D printing emission compared to the air only controls. This finding was supported by increased OVA-specific IgE levels and alterations in lung pathology in exposed mice. In comparing the cellular phenotyping of BALF, the 3D printing group showed significant increases over the air control in the number of CD11b+ monocytes and macrophages and in mean fluorescence intensity (MFI) of CD86 on B cells. In addition, a significant increase in MFI of CD86 on alveolar macrophages in lungs was observed. These results were supported by changes in lung tissue gene expression and cytokines analyses. **Conclusions:** The findings from this study suggest that emissions from 3D printing with polycarbonate filament results in enhanced airway reactivity and support the need for additional investigation in this area of research.

SOT 63RD ANNUAL MEETING & TOXEXPO
SALT LAKE CITY, UTAH • MARCH 10–14, 2024

The Toxicologist

Supplement to *Toxicological Sciences*

SOT | Society of Toxicology

Toxicological Sciences

The Official Journal of the Society of Toxicology

OXFORD UNIVERSITY PRESS

ISSN 1096-6080 Volume 198, Issue S1 (March 2024)
www.academic.oup.com/toxsci

Publication Date: March 5, 2024