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Suppression of Acute Respiratory Distress Syndrome Pulmonary Inflammation by Transfer Factor Activated Dendritic Cells: StemVacs™

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Abstract:

Despite falling into disfavor amongst immunologists as a means of antigen-specific immune modulation, the potential of Transfer Factor (TF) as to act as an inducer of innate immunity has not been evaluated. Utilizing leukocytes from healthy donors, we generated leukocyte lysate employing a procedure similar to that used in preparation of TF with exception that the donors were not pre-immunized. We demonstrated superior augmentation of costimulatory molecules, cytokine production and T cell activation by dendritic cells treated with TF as compared to those activated using conventional protocols. Administration of TF-activated allogeneic dendritic cells resulted in suppression of B16 melanoma growth in an NK dependent manner. Interestingly administration of the cells in a murine model of ARDS resulted in suppression of pulmonary pathology. Given that StemVacs has already been used in humans and is subject of an FDA IND application, utilization of this cellular therapy for treatment of COVID-19 may be considered.

Background

One of the major causes of COVID-19 associated fatality is Acute Respiratory Distress Syndrome (ARDS). This is a condition of acute respiratory failure caused by a variety of factors which are related to inflammation and release of various activators of the innate immune system such as cytokines and inflammatory factors [1-9]. An ideal therapy would involve stimulation of immunity while suppressing inflammation.

It is widely known that ARDS generally presents with progressive hypoxemia, dyspnea and increased work of breathing. Patients often require mechanical ventilation and supplemental oxygen [10]. Over the years, our understanding of ARDS has advanced significantly, with elucidation of several of the molecular and cellular pathways involved in initiation, progression and resolution/fibrosis. However, ARDS is still represents significant morbidity and mortality and therapeutic strategies to mitigate the foregoing have resulted in limited translational success. Part of this failure stems from the very different presentations of ARDS between people, as well as differences in their genetic composition.

ARDS is caused by many situations bacterial and viral pneumonia, sepsis, inhalation of harmful substances, head, chest or other major injury, burns, blood transfusions, near drowning, aspiration of gastric contents, pancreatitis, intravenous drug use, and abdominal trauma. Furthermore, those with a history of chronic alcoholism are at a higher risk of developing ARDS [11-13]. Alcoholism affects several parameters relevant to ARDS including: a) reduction in glutathione levels [14, 15], b) increasing levels of adhesion molecules on lung blood vessels so as to increase recruitment of inflammatory cells [16]; c) upregulating lung adenosine levels, resulting in impaired active Na(+) transport in the lung [17]; and d) suppression of pulmonary immunity [18].

One of the cardinal symptoms of ARDS is fluid accumulation in the lungs. When this occurs, the elastic air sacs (alveoli) in the lungs fill with fluid and the function of the alveoli is impaired. The result is that less oxygen reaches the bloodstream, depriving organs of the oxygen required for normal function and viability. In some instances, ARDS occurs in people who are already critically ill or who have significant injuries. Severe shortness of breath, the main symptom of ARDS, usually develops within a few hours to a few days after the precipitating injury or infection. [19]
Unfortunately, many patients who develop ARDS do not survive. The risk of death increases with age
and severity of illness. Of the people who do survive ARDS, some recover completely while others
experience lasting damage to their lungs.

Currently there exist no effective pharmacologic therapies for treatment or prevention of ARDS. While
inhibition of fibrin formation mitigated injury in some preclinical models of ARDS, anticoagulation
therapies in humans do not attenuate ARDS and may even increase mortality. Protective lung ventilator
strategies remain the mainstay of available treatment options. Due to the significant morbidity and
mortality associated with ARDS and the lack of effective treatment options, new therapeutic agents for
the treatment of ARDS and new treatment methods for ARDS are needed.

The current paper examined whether allogeneic dendritic cells (DC) can be utilized for stimulation of NK
mediated immunity, while concurrently not exacerbating ARDS. The possibility of a dual NK stimulation
and ARDS suppression is intriguing.

**Materials and Methods**

**Mouse Model**

Female C57BL/6 mice of 8 weeks of age were inoculated with 1 million B16 cells in the flank. Mice were
administered allogeneic BALB/c bone marrow derived dendritic cells which were not antigen pulsed. For
experiments involving lung fluid content, BALB/c mice were administered 5 ug of lipopolysaccharide
intratracheally and sacrificed at the indicated timepoints. DC were administered intravenously via tail
vein injection. Lung fluid content was measured by weight the lung versus the weight of the body.

**Cell Cultures**

Whole blood (20–50 ml) from healthy donors (>80 participants) was mixed with Ficoll-Hypaque, and
after centrifugation the layer of mononuclear cells was collected. After lysis of RBC, the mononuclear
cells were laid on petri dishes (Costar, Cambridge, MA) for 30–60 min at 37°C to remove nonadherent
cells. After five washes with PBS, adherent cells were cultured in 3 ml of medium containing 800 U/ml
GM-CSF and 500 U/ml IL-4. The culture medium was changed every other day with 300 μl of fresh
medium containing 2400 U of GM-CSF and 1500 U of IL-4. The detached cells, the main population of
CD1a+ cells (our unpublished observations), were used for experiments after culture for 7 days.
Consistently, >95% of the cells in the gated region expressed CD1a.

Maturation of DC was performed by culture with 10ng/ml lipopolysaccharide and 10 ng/ml TNF-alpha.
Assessment of maturation was performed by flow cytometry for CD80, CD86 and IL-12 production.

Assessment of natural killer cell activity as performed using ProMega cytotoxicity kit as per
manufacturers instructions.

**Cytokine Analysis**

Standard ELISAs were used to measure cytokine concentrations in harvested. All determinants were
performed triplicate (IL-12) and expressed as the mean ± SD. The expression of cell surface molecules
was determined by flow cytometric analysis. Each histogram or density plot comprised at least 104
events and expressed as mean fluorescent intensity.

**Transfer Factor**
Peripheral blood leukocytes were resuspended in 5 ml sterile water (Lonza). Cells were frozen and then thawed using dry ice and 100% ethanol bath, alternating with a 37°C water bath. The freeze-thaw cycle was performed at least 7 times. The product was then placed into dialysis bags with an 8 kDa cutoff that had been boiled for 3 intervals of 20 min (Spectrum Labs, Rancho Dominguez, CA, USA) against 50 vol water. Dialysis was conducted for 24 h at 4°C under constant stir. Dialysis was repeated for an additional 24 h with an identical volume of water after the first amount was removed and frozen. Subsequent experiments using ultrafiltration were performed by filtering lysed splenocytes through a Centriprep-10K cutoff ultrafiltration unit (EMD Millipore, Billerica, MA, USA). After the second dialysis or after ultrafiltration, the contents from both dialysis sessions were combined in T225 flasks (BD Falcon; BD Biosciences, San Jose, CA, USA), frozen to −80°C, and then lyophilized (FreeZone 2.5; Labconco, Kansas City, MO, USA). Lyophilized remnants were resuspended in sterile water or DMEM (Thermo Fisher Scientific) to a cell-equivalent concentration, as indicated.

Results

Induction of Dendritic Cell Maturation

Transfer Factor (TF) was generated according to previously published protocols and added to dendritic cell progenitors. As seen in Figure 1, dendritic cells activated with TF possessed higher expression of CD80 (Fig 1a), higher expression of CD86 (Fig 1b), lower expression of IL-12 (Fig 1c) and possessed enhanced ability to stimulate NK cytotoxicity (Fig 1d).

Figure 1: Transfer Factor Activates Dendritic Cells
Allogeneic Non-pulsed Dendritic Cells (StemVacs™) Inhibit B16 Melanoma in an NK Dependent Manner

It is well-recognized that NK cells play a role in suppression of cancer and metastasis [20-22]. Studies have shown that in certain situations non-pulsed DC are capable of eliciting an anticancer response through stimulation of NK cell therapeutic activity [23-26]. The importance of DC to cancer can be seen in numerous studies showing superior prognosis in patients having dendritic cell infiltrations in the tumor [27, 28]. In order to assess whether StemVacs™ allogeneic TF activated DC are capable of inhibiting tumor growth, we administered cells in a wild-type B16 models (Figure 2a) and in NK depleted mice (Figure 2b). Strikingly, the antitumor activity was diminished in absence of NK cells. This indicates the possibility that allogeneic TF pulsed DC may be useful as a cancer therapeutic.

Suppression of Lung Inflammation by StemVacs™

One therapeutic possibility for cell therapies which activate NK cells could be in the treatment of COVID-19. One of the potential drawbacks of utilizing immunotherapies in this condition is the possibility of cytokine storm and exaggeration of pulmonary failure. Accordingly, we sought to assess whether administration of StemVacs™ would exacerbate inflammatory reactions subsequent to endotoxin challenge. To our surprise StemVacs™ administration was associated with enhanced protection from lung inflammation as compared to conventionally activated DC which actually enhanced inflammation (Figure 3).
Discussion

We have demonstrated that transfer factor activated DC are capable of inducing potent immunity by stimulating both T and NK cells. The current data support the translational use of StemVacs™ in treatment of COVID-19 due to its unique ability to activate NK cells but also suppress inflammation in the lungs.

Pulmonary inflammation such as in ARDS is associated with augmentation of interleukin-17 [29, 30]. It is known that the cells which produce IL-17, called Th17 cells, have a reciprocal relationship with T regulatory cells. Indeed, it may be possible that our dendritic cell therapy was stimulating NK cells which account for its anticancer properties, while stimulating T regulatory cells, which may have been responsible for reduction of inflammation.

In many situations it is established that mature DC activate T cells while immature ones generate T regulatory cells. Two of the authors (WPM and TEI) more than ten years ago described the Tolerogenic Feedback Loop in which permanent allograft tolerance is maintained through a self-amplifying loop between regulatory T cells and tolerogenic dendritic cells [31]. We believe that further experiments are needed to determine whether the suppression of pulmonary inflammation is occurring as a result of T regulatory cells being elicited.

While utilization of autologous dendritic cells is well-known in the area of oncology, allogeneic dendritic cells present the tantalizing option of an “off the shelf” therapy. Initial clinical reactions using this approach have demonstrated safety (manuscript in preparation) and signals of efficacy. It may be that allogeneic dendritic cells work by activating NK cells as compared to T cells. Further studies are needed to elucidate whether it is the allogeneic nature of the stimulating cells, or whether it is the potency of the TF in activating DC.

One unexplained observation was the finding that while TF stimulated expression of costimulatory molecules in a manner superior to LPS and TNF-alpha, it did not stimulate IL-12 production. IL-12 is needed for NK activation, however NK activation was higher with TF as compared to traditional means of activating dendritic cells. We postulate that other cytokines may be involved such as IL-18, which we are in the process of assessing.

In conclusion, StemVacs™ appears to be a novel means of stimulating innate immunity while concurrently suppressing cytokine storm and pathology associated with adaptive immunity. These current data will serve to provide preclinical support for the planned StemVacs™ IND application.
References


