Immune Gene And Cell Therapy: A Novel and Promising Approach for Immunotherapy of Sepsis Management

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Aim and Background (Necessity of the project):

As a host systemic response to invasion of microbial pathogens into bloodstream, "Sepsis" has been considered as one of the leading cause of death in critically ill patients especially for the patients hospitalized in the Intensive Care Units of the developing countries. Approximately, 250,000 people succumb from sepsis in the USA, annually and almostly 2% of them are reported as severe septic patients with often bacterial resource. Pneumoniae, abdominal and urinary infections are most common types of clinical manifestations in mentioned patients.

Common therapeutic strategies for sepsis are: supportive treatments, revised nutritional regimens, surgery of inflamed site and antibiotic therapy. On the one hand, these approaches have not been definitely beneficial for all cases, yet. On the other hand, indiscriminate usage of antimicrobial drugs, antibiotic resistance, predisposition infections or genetic composition of patients, reappearance of invasive pathogens with chronic incubation period and changing nature of clinical manifestations of the disease, all are inevitable barriers that make clinical diagnosis and treatment, inaccessible.

These issues encourage basic medical scientists to put up with a barrage of questions in order to conduct investigations and more focus on comprehensive attitudes related to sepsis immune-pathophysiology. It seems that it is rational to adhere such principles in order to diminish organ failure and mount concerns over sepsis related mortalities of such global crisis.

Stages in sepsis divide into 4 levels. To summarize, Systemic Inflammatory Response syndrome (SIRS) andCompensatory Anti-inflammatory Response Syndrome (CARS) are of high importance. Accumulation of antiinflammatorycytokines leads to immunosuppression induced mortality. So, it seems that restoration of sepsisinducedinflammation, will be an acceptable therapeutic strategy.

Anti-Macrophage Inhibitory Factor (Anti-MIF), PD-1 blocker, Interleukin-7 (IL-7), IL-12, IL-15 and GMCSF based immunotherapies have been recently introduced to clinical trials.

Surprisingly, during last decade, exponential growth of approaches with immunological perspectives such as special sero-immunobiomarkers, stem cells and vectors with easy accessibility, less morbidity and operatively yields in clinical trials, have opened a new window to clinical applications.

Hypothesis

For the first time in this hypothesis article, we propose inflammatory effects of IL-33 gene transfer via Adenoassociated Virus (AAV) as a vector by Bone Marrow derived Mesenchymal Stem Cells (BM-MSCs) in a Cecal Ligation and Puncture (CLP) murine (C57BL/6) model, in sepsis global dilemma domination.

1. Various evidences suggest that IL-33 (IL-1F11) has important roles in immune system regulation, and promote the resolution of inflammation.

One of the main criteria that should be met is the fundamental immune-biology of IL-33, is that it is dependent to function of ST2L (also known as T1, IL-1RL1, DER4) that leads to recruitment of MyD88 and activation of NF-Ⅱ via it`s cascade chain in order to product inflammatory mediators. Hence, IL-33 can be used as an immunomodulatory agent in the sepsis gene therapy.

2. Adult MSCs, are a population of cells with a wide range of capabilities as: self-renewability and differentiation into mesenchymal tissue (osteocytes, chondrocytes, or adipocytes).

The immunomodulatory properties of MSCs involved in: anti-inflammation, anti-bacterial, anti-oxidant, anti-apoptotic and metabolomics effects. In sepsis, immunomodulation of IFN-Ⅱ and HLA-G5 stimulated MSCs, appears as suppressor for proliferation and activation of T-cells and simultaneous activation of Tregs through indoleamine 2,3-dioxygenase and prostaglandin E2. Thereafter, increased secretion of IL-10, inhibition of antigen presentation by Dendritic Cells (DCs), antitumor immunity due to production of CD73+ Natural Killer Cells (NKCs), inhibition of Th1 differentiation into Th17, are expectable.

3. Various viral and non-viral vectors have been modified in gene and cell based therapy applications including Retrovirus, Adenovirus, AAV, Lentivirus and Herpes Simplex Virus (HSV). As a DNA virus non-pathogenic vector, ΔE1ΔE4 Adeno-Associated virus (AAV) vectors are more suitable than other types of gene delivery vehicles without causing immuno-genotoxicity properties. AAV can be used for delivering therapeutic transgenes as cytokines for stimulating the immune system. Due to their high prolonged transgene expression (transduction efficacy), expected ability and safety in sepsis immune-pathogenesis, AAVs in this hypothesis are suggested.

4. No hesitate that it is needed a suitable animal model for pre-clinical studies of this hypothesis however they may be potentiated to re-generalize into human studies. Sepsis clinical manifestations are mainly clarified in the lungs or peritoneal cavity, therefore CLP murine model of bacterial peritonitis can be served as gold standard model of sepsis due to its inflammation induction capabilities, reproducibility, affordability and being user-friendly.

Evaluation Of The Hypothesis

To evaluate this hypothesis, we propose following levels to be assessed. It is of high prominence to mention that all of the injections are done subcutaneously (except for MSCs that are injected intravenously).

- **Control** group**:** (n=10) induction of syngeneic CLP murine models (receiving no injections).
- **First** case group: (n=10) transfection of separated MSCs by IL-33 gene delivery through lipofection method.
- **Second** case group: (n=10) transduction of IL-33 gene into AAV.
- **Third case** group: (n=10) transduction of separated MSCs and empty AAV.
- Transfusion of mouse's modified gene MSC to own blood stream and consideration of time for injection.
- Isolation of MSCs: carrying out from CLP mouse model BM, centrifuge and subsequent culture of supernatants, consideration of time and temperature for incubation.

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- Serum IL-33 levels detection by Enzyme Linked Immunosorbent Assay (ELISA) for each group.
- IL-33 gene expression by Real-time Polymerase Chain Reaction (PCR) for each group.
- Investigation of systemic vascular resistance and cardiac output.
- Investigation of IL-10 expression as a marker of attenuated sepsis-induced multi-organ dysfunction.
- Investigation of kidney, liver and pulmonary functions and CNS and haematological alterations.
- Investigation of plasma biomarkers, i.e.: IL-6 Complete Blood Count with differential.

Conclusion

We hope that pro-inflammatory effects of IL-33 gene transfer via AAV as a vector in a CLP mouse model, will probably be efficient in activation of pro-inflammatory cytokines and reduction of immunosuppression-induced mortality. To achieve this, further investigations and more collaborations between cell biologists, immunologists, laboratory scientists, infectious diseases and internal medicine specialists are unquestionably needed.