Impact of media supplementation on the secretion of IFN-γ induced indoleamine 2-3 deoxygenase and resultant immune suppression by mesenchymal stromal cells

Peggy Bulur, Timothy D. Wiltshire Ph.D., Amel Dudakovic*, Ph.D., Andre J. van Wijnen* Ph.D., Allan B. Dietz, Ph.D.

Division of Transfusion Medicine and Experimental Pathology, Immune Progenitor and Cell Therapeutics (IMPACT) Lab, Departments of Orthopedic Surgery* or Laboratory Medicine and Pathology, Mayo Clinic, Rochester, MN

Abstract

Mesenchymal Stem Cells (MSC) have demonstrated significant therapeutic utility in a number of indications. The multi-functional nature of these cells includes significant capacity to suppress immune responses. Little is known regarding the mechanisms that induce or maintain the immune suppressive phenotype desired for some clinical applications. Two suggested methods for enhancing the immune suppressive function of MSC is to pre-incubate the cells with IFN-gamma or the use of fibrinogen reduced media supplement. Functional characterization of immune suppression is tricky, relying on development of standard assays prior to use in a clinical environment. Indoleamine 2-3 deoxygenase (IDO) is a well known clinical target molecule that catalyzes tryptophan arizing T and NK cell function and enhancing peripheral homeostasis. It easily measured with a large dynamic range. It has been extensively studied in vivo with expression of IDO directly correlated to the level of systemic immune suppression. As such IDO secretion allows a rapid and sensitive release assay. We split healthy donor adipose tissues and cultured them in multiple conditions to generate matched MSC samples derived in a homogeneous environment. We measured growth kinetics for all samples. Samples were also submitted for RNA-Seq to determine their steady state immune suppression profile. We measured growth kinetics and native IDO secretion. IFNγ induced IDO expression is dependent on IFNγ concentration, the time of exposure, the donor, and the media supplement used to culture the MSC. Doubling the activation time from 24-48 hours approximately doubled the IDO expression. Together, this data suggests that measurement of IDO secretion is a sensitive and rapid test that may be useful for release testing, that IFNγ can be used to enhance the secretion of IDO, and that there may be substantial variability in the level of IDO secretion due to donor and the media supplements used to grow the MSC.

Methods

IFNγ incubation time drives IDO Production

24 hour Licensing of MSC

48 hour Licensing of MSC

Effect of Media Supplementation on Cell Kinetics and IDO expression

IDO as a marker of immune suppression

Population doublings are consistent within donors. MSCs were cultured in media with designated supplement and population doublings calculated to determine cell kinetics. Cells were licensed with IFNγ for 24 hours and media washed out. New media without IFNγ was added back. IDO secretion time indicated hours was measured with an IDO ELISA.

Effect of culture conditions on expression of select immune secretome

Fat samples from different donors (n=4) were split and grown as MSC in various culture conditions (n=5). Cells were collected at approximately the same number of doublings prior to confluence. RNA was isolated and used for RNA sequencing. Chemokines and cytokines were selected with robust average expression and clustered using Morpheus (Broad Institute). Copying from different donors (n=4) were split and grown as MSC in various culture conditions (n=5). Cells were collected at approximately the same number of doublings prior to confluence. RNA was isolated and used for RNA sequencing. Chemokines and cytokines were selected with robust average expression and clustered using Morpheus (Broad Institute).

Conclusions

- With the exception of FBS, donor MSC population growth kinetics were similar when used as directed.
- No conditions demonstrated detectable IDO expression in the native cultures.
- IFNγ was a powerful inducer of IDO, but this induction was dependent on IFNγ concentration, the time of IFNγ exposure, the donor, and the media in which the cells were grown.
- Intra-assay IDO expression was highly reproducible within a donor, however there was substantial donor to donor variation.
- IFNγ licensing improved with culture period extended to 48 hours.
- After IFNγ washout, expression of IDO continued to increase for 48 hours and then plateaued for up to four more days of culture.
- Culture conditions profoundly affected the amount of IFNγ induced IDO secretion in different cultures and may allow for a method to identify those donors that may be better suitable for allogeneic protocols needing immune suppression – making them a potential powerful release assay. In addition, IFNγ licensing is an in vitro mimic of the in vivo condition the cells will be placed. This data strongly suggests that culture environment plays a profound impact on the therapeutic index of the cells.

Together, this data demonstrates that IFNγ induced IDO expression is highly reproducible within samples and may allow for a method to identify those donors that may be better suitable for allogeneic protocols needing immune suppression – making them a potential powerful release assay. In addition, IFNγ licensing is an in vitro mimic of the in vivo condition the cells will be placed. This data strongly suggests that culture environment plays a profound impact on the therapeutic index of the cells.

Effect of Media Supplementation on Cell Kinetics and IDO expression

Media supplement impacts donor production of IDO in response to low (3ng/ml) or high (50ng/ml) IFNγ concentrations. Donor MSC were licensed with IFNγ for 48 hours and media washed out. New PLTGold media without IFNγ was added back. IDO secretion over 48 hours was measured (IDO ELISA).

Media supplement impacts donor production of IDO in response to low (3ng/ml) or high (50ng/ml) IFNγ concentrations. Donor MSC were licensed with IFNγ for 48 hours and media washed out. New PLTGold media without IFNγ was added back. IDO secretion over 48 hours was measured (IDO ELISA).

Conflict of Interest

All authors have declared conflict of interest. All authors have participated in the study design, data analysis, and manuscript preparation. All authors have read and approved the final draft.