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

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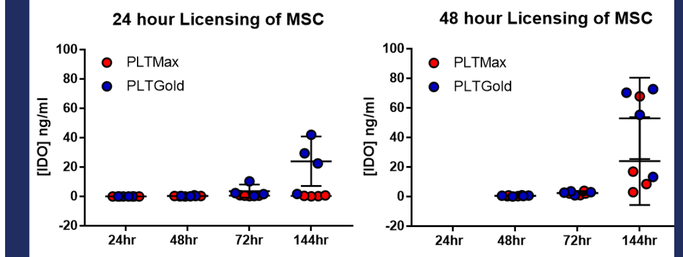
Abstract

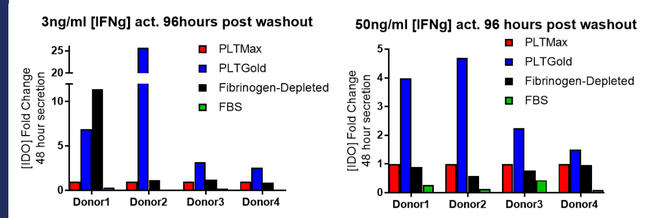
Mesenchymal Stem Cells (MSC) have demonstrated significant therapeutic utility in a number of indications. The multi-functional nature of these cells includes significant capacity to suppress immune responses. Little is known regarding the mechanisms that induce or maintain the immune suppressive phenotype 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 catabolizes tryptophan arresting 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.

IFNγ incubation time drives IDO Production



Licensing time impacts IDO production. MSC from 4 donors are cultured in either PLTMax supplemented or PLTGold supplemented media and IFNγ licensed for 24 or 48 hours. IFNγ containing media is washed out and replaced with designated media at listed time points. IDO measured in culture supernatants at listed time points.

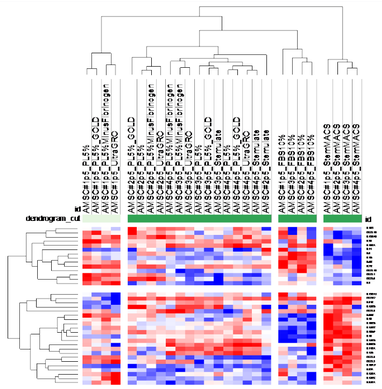
The effect of IFNγ concentration and media composition on IDO expression



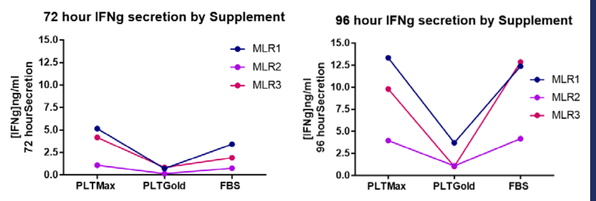
Media supplement impacts donor production of IDO in response to low (3ng/ml) or high (50ng/ml) IFNγ licensing. Donor MSC were licensed with IFNγ for 48 hours. IFNγ containing media was washed out and replaced with designated supplemented media every 48 hours. Cells are cultured an additional 96 hours. IDO is measured by ELISA on 96 hour post washout supernatant.

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).



Impact of media supplement on MLR IFNγ production



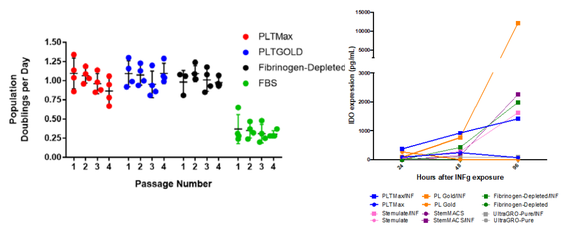
Media supplement impacts IFNγ production in MLR. Equal numbers of PBMC (3 donors each MLR, using 1.5e6 /2ml/well) were co-cultured for 96 hours in designated supplemented media. IFNγ was measured by ELISA.

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 effected the amount of IFNγ induced IDO secretion. PLTGold supplemented media was up to three times better than a standard PL supplement and almost ten times better than other conditions. There was no observed consistent advantage of fibrinogen depletion for IDO expression.
- Media supplement used in MLR impact the level of IFNγ expression, thus effecting the interpretation of immune suppressive amount.
- In preliminary studies, RNA-SEQ suggests profound donor and culture dependent differences in immune related molecules.

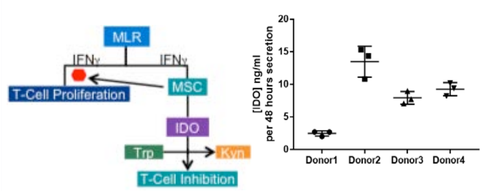
Together, this data demonstrates that IFNγ induced IDO expression is highly reproducible within samples and may allow for a method to identify those donors that may be better suitable for allogeneic protocols needing immune suppression – making them a potential powerful release assay. In addition, IFNγ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

Effect of Media Supplementation on Cell Kinetics and IDO expression



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).

IDO as a marker of immune suppression



IDO as a measurement of immune suppression and its consistency within Donor. Individual donor MSCs were plated (n=3) times in PLTGold supplement media. Cells 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).

Methods

- MSC were derived from healthy donors fat tissue with approval from the Mayo Clinic IRB. Fat tissue was minced and digested with Collagenase I solution at 37◦C. Digested samples were centrifuged to pellet cells, re-suspsended in Advanced MEM containing 1% GlutaMax and 1% Penicillin/Streptomycin and one of the following supplements: PLTMax, PLTGold (both MillCreek Life Sciences, Rochester, MN), Human Platelet Fibrinogen Depleted (StemCell Technologies, Seattle, WA), and FBS (MediaTech, Manassa VA; Stemulate, Cook, Indianapolis, IN, UltraGRO, AventaCell, Atlanta, GA StemMACS MSC Expansion Media Kit XF, human, Miltenyi Biotec, Cologne, Germany). All supplements at 5% v/v. Cell suspensions filtered through 70 then 40µm filters. Cells were pelleted and suspended in designated supplemented media. Media was replaced with designated new media every 2-3 days. Cells were allowed to proliferate 5-7 days before being passaged, designated here as p0. MSC cell lines were maintained in original starting culture media throughout passages. After p0, cells were seeded at 3000 cells/cm2 standard. Cultures were maintained at 37◦C, 5% CO2 humidified incubator for 3-4 days and passaged at 80-95% confluence.
- In all cell culture conditions, MSC were used in assays after an initial 3 day return to culture from frozen stocks.
- PBMCs were freshly isolated from Trima Cones procured in the Mayo Clinic Components Laboratory. Fresh PBMC are most representative of cells in the body. Monocytes from frozen PBMC lose viability during the freeze/thaw processing.

Conflict of Interest

Allan B. Dietz is an inventor of technology used as a tool in this research; the technology has been licensed to a commercial entity (PLTMax; Mill Creek Life Sciences). ABD and Mayo Clinic have equity in the company. ABD has contractual rights to receive royalties from the licensing of this technology. ABD has governance responsibilities within this company. These conflicts have been disclosed to and are managed by the Mayo Clinic Conflict of Interest Board and are included here as directed by them.