REPORT

Report on participation of the ICMR International Fellow (ICMR-IF) in Training/Research abroad.

1. Name and designation of ICMR- IF : Dr Salauddin Qureshi, Pr. Scientist

2. Address : Division of Biological Standardization, ICAR-IVRI, Izatnagar

3. Frontline area of research in which training/research was carried out :

Treg cells constitute 5–10% of total peripheral T cells in mice as well as in humans. CD4 + Tregs have a role in maintaining immune homeostasis and preventing autoimmune reactivity. Treg cells also regulate other effector T cells functions. The majority of Treg cells are generated in the medullary region of the thymus gland as single positive CD4 T (CD4-SP) cells. Medullary thymocytes expressing higher affinity interactions with different transgene encoded antigens are required for the development of Treg cells while lower affinity TCR do not have the ability to differentiate into Treg cells. For proper development and function of Treg cells, Tregs are crucially depend on the forkhead box transcription factor FOXP3; loss of Foxp3 function in humans and rodents results in devastating autoimmunity. A vast majority of Foxp3 + Tregs are generated during T cell development in the thymus.

Plasticity of T helper cells has been well documented, and especially, Th17 cells were reported to acquire Th1 phenotypes. Some of the Th17 cells present in Crohn's disease were able to produce both IL-17 and IFNγ, and it been suggested that some of these proinflammatory Th17 cells may act like the Th1 type as well. CD4 T cells co-expressing IL-17 and IFNγ (IL-17 + IFNγ +) are strongly associated with plasticity of T effector toward Treg cells and have a capacity to tip the balance toward T-cell regulation. Interventions using elF5A inhibition with GC7 of CD4 T cell subsets (T helper and Treg) resulted in amelioration of T1D but was not able to revert T1D, at least in our humanized T1D mouse model until interventions like anti-DLL4 restrained autoreactive CTLs in the islet microenvironment. With this I started the work to study the role of combined inhibition of Notch/ elF5A pathways to reset the immune imbalance by inducing plasticity of effector cells towards regulatory T cells. The work on validation of immunogenomic

role of elF5A inhibitor: N1-guanyl-1,7-diaminoheptane (GC7) and Notch inhibitor: anti-DLL4 Mab, in modulating immune responses and promoting regulatory T cells.

Work plan:

a. The investigation of plasticity of CD4+ and CD8+T cells isolated from PBMCs of latent autoimmune diabetes of adult (LADA) patients (n=3) and nondiabetic healthy donors (n=4) were carried out in ex vivo experimentation.
b. The sorted CD4+ and CD4+ deficient T cell populations from PBMCs were cultured separately in media supplemented with GC7 (100 μM)+anti-DLL4 (10 μg/ml)+ rhGAD65 (4 μg/ml) and compared with conventional stimulation by anti-CD3 /CD28dyna beads for 7 days.
c. To dfine the origin of plasticized Tregs we designed next experiment creating a pool of Treg deficient CD4+ T cells (CD4+CD25-) and CD4+ deficient T cells and traced the lineage of plasticizing cells. Both cell populations were cultured in media supplemented with GC7 +anti-DLL4 + rhGAD65 and compared with conventional stimulation by anti-CD3 /CD28dyna beads and control media.
d. The quantification of plasticized CD4+CD25+ cells out of total CD4+CD25- cells were done by flow cytometry.
e. To access the functional phenotype of plasticized Tregs (CD4+CD25+), we designed a suppression assay with plasticized CD4+CD25+ and freshly isolated Tresp (CD4+CD25-) cells from PBMCs. We evaluated the suppression capacity of plasticized cells (CD4+CD25+) by co-culturing with freshly isolated autologous Tresp cells in Treg:Tresp ratios of 0:1, 1:1, 1:2 and 1:0 for 5 days.
f. For assessment of cell viability and any apoptotic/ off target effect due to immunomodulatory treatment we quantified the live, dead, and apoptotic cells at 24h, 48h, 96h, and 7 days interval using membrane permeable dead cell apoptosis kit.

4. Name & address of Professor and host institute : Dr Shanawaz Imam, Assistant Professor, Department of Medicine, College of Medicine and Life Sciences, University of Toledo, Toledo, Ohio, USA

5. Duration of fellowship with exact date : 30th March 2023 to 26th July 2023

6. Highlights of work conducted :

   i) Technique/expertise acquired :

a. Immune cell profiling and proliferation assay by flow cytometry, ELISA, RNA-seq analysis, confocal microscopy
b. Isolation of islets of Langerhans
c. Colorimetric / fluorometric assays of cholesterol, LDL, HDL, Triglyceride, Albumin and creatinine from serum and urine samples

ii) Research results, including any papers, prepared/submitted for publication :

a. Tregs were co-cultured with T effector cells and suppression/proliferation was accessed after 5 days. To create an ex-vivo Treg deficient environment, we sorted out CD4+ cells from the PBMCs.
b. CD4+ deficient PBMCs and sorted single positive CD4+CD25- cells were treated with anti-DLL4+GC7+rhGAD65 with and without IL-2 supplementation and were compared with conventional anti-(CD3+CD28) stimulation and absolute control (20% FCS+RPMI).
c. The presence of Treg cells was confirmed by flow cytometry. 30.5±5.1% of CD4 cells were plasticized into Tregs in the treatment group supplemented with IL-2 while only 18.8±4.6 % were plasticized in treatment group without IL-2 supplementation.
d. Also, 11.2±3.1% plasticized to Tregs in treatment group in presence of CD3/CD28 microbeads compared to 6.19±2.4% in absolute control group.
e. Similarly, 13±4.3% of CD8 cells had plasticized into Tregs in treatment group supplemented with IL-2 while only 11.5±6.7% plasticized in treatment group without IL-2 supplementation. 14.3±5.8% plasticized to Tregs in treatment group in presence of CD3/CD28 microbeads compared to 12.4±6.3% in absolute control group.
f. The plasticized Tregs from LADA patients were more robust than those of non-diabetic patients at a 1:2 ratio (Treg:Tresp).
g. The plasticized Tregs had comparable suppressive capacity over Tresp (CD4+CD25-) cells as that of freshly isolated naïve human Tregs.
h. To investigate any off-target effects of (GC7 and anti-DLL4), live, dead, and apoptotic cell count at 24, 48, 96 hours, and 7 days post-treatment using dead cell apoptosis kit (Invitrogen). There was no significant difference between treatment and control groups in terms of live, dead, and apoptotic cells at different time intervals. On the 7th day of culture 64.06±8.66% and 68.88±6.15% of cells were live in treatment and absolute control groups respectively while 7.24±1.59% and 10.0 ±2.02% cells were found apoptotic respectively.
i. Therefore, we define the reprogramming of T effector cells towards Treg cells with functional suppressive capacity using GC7 and anti-DLL4 without any off-target effect (toxicity).

iii) Proposed utilization of the experience in India :

This training provides sufficient information and knowledge to understand the basics of immune cells plasticity and immunogenomic approach to study the immunomodulation and immunomonitoring of infectious diseases.

22/12/23

Signature of ICMR-IF

ICMR Sanction No. No. INDO/FRC/452/(S-35)/2022-23-IH&HRD, 19.10.2022

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