REPORT

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

1.	Name and designation of ICMR- IF	: DR PRASENJIT DAS
2.	Address	: DEPARTMENT OF PATHOLOGY. ROOM 1083, TEACHING BLOCK. AIIMS. NEW DELHI 110049
3.	Frontline area of research in which training/research was carried out	: GASTROINTESTINAL DISEASES
4.	Name & address of Professor and host institute	: PROF JERROLD R TURNER. BWH, HARVARD MEDICAL SCHOOL. 7TH FLOOR. 77 AVN LOUIS PASTEUR, BOSTON MA 02115-5727
5.	Duration of fellowship with exact date	: 21/03/2023 TILL 20/6/2023
6.	Highlights of work conducted	:

i) Technique/expertise acquired :

1) Learned and standardized multiplex immunofluorescence staining method.
2) Learned to analyze multiplex staining by using Image J and Cell Profiler softwares.
3) Learned basic protocol of mouse model studies- maintaining mouse colonies, methods of inducing colon carcinoma and intestinal inflammation and harvesting of tissue for further analyses.
4) Observed and learned protocol for intestinal permeability assay and intestinal transepithelial resistance studies.
5) learned the basic principles of using multiple immunofluorescence stains together on archival tissues

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

I worked on two projects during my 3 months attachment with Professor Turner's lab.

I] In this study I worked with archived intestinal tissue of various models of mouse colitis models and compared with changes in human IBD to understand how different mouse colitis models histologically differ from that of human IBD. This was important as mouse colitis models have been utilized for decades as a crucial preclinical tool to comprehend the pathophysiology of diseases and to evaluate drugs for human inflammatory bowel disease (IBD). We have demonstrated that despite numerous similarities, there are still differences that exist. Not all in vivo experimental models can be considered representative of human IBD.

Papers: I completed the work and submitted the full manuscript to Prof Turner for his input. it will be sent for publication when work up completes. acknowledgement for ICMR-DHR Fellowship has already been included.

II] Study of molecular changes associated with small bowel enteropathies from patients with a) diagnosed 'celiac disease' (n 100) and b) 'non-celiac enteropathies' (n=100), comprising of cases with giardiasis, cryptosporidiosis, Crohn's disease, Common variable immune deficiency, potential celiac disease, non-celiac gluten sensitivity (NCGS), and first-degree relatives of patients with celiac disease using the multiplex immunofluorescence staining of tissue-microarray sections, and widefield and stimulated emission depletion (STED) super-resolution microscopy, and image morphometry.

In this work we first made tissue microarray slides and then established the sequential multiplex immunofluorescence staining protocol. In this protocol one tissue section was stained with multiple IF tagged antibodies and thereafter bleaching. We used Scanning scope to digitize all ROIs and stitched them. I learned to use Image J and Cell profiler softwares and writing pipelines to analyze the scanned data. As the scanning, staining-bleaching and restaining took an enormous time I could generate data from this work. and I brought data with me and am doing analyses now. Writing pipelines in Cell profiler software and automated analyses takes long time on scanned images and it may take another 2 months' time to complete the whole work and analyze final data and writing manuscript.

Professor Turner was extremely generous and helpful to let me use his laboratory set up, learn this valuable technique and use all IF antibodies. the staining sequence and markers

used are as follows and they are much more than what we proposed initially to understand the pathological changes in small bowel diarrheal diseases better:

SET1	1 (TS)	2 (CD)	3 (OE)	4 (NCGS)	5 (GD)
R1	DAPI	DAPI	DAPI	DAPI	DAPI
R2	CD8CD3NaKgActin	CD8CD3NaKgActin	CD8CD3NaKgActin	CD8CD3NaKgActin	CD8CD3NaKgActin
R3	DAPI	DAPI	DAPI	DAPI	DAPI
R4	CD8CD3NaKFoxp	CD8CD3NaKFoxp	CD8CD3NaKFoxp	CD8CD3NaKFoxp	CD8CD3NaKFoxp
R5	FoxP3CD3CD20NaK	FoxP3CD3CD20NaK	FoxP3CD3CD20NaK	FoxP3CD3CD20NaK	FoxP3CD3CD20NaK
SET2					
R1	DAPI	DAPI	DAPI	DAPI	DAPI
R2	Cl2Cl4Zo1gActn	Cl2Cl4Zo1gActn	Cl2Cl4Zo1gActn	Cl2Cl4Zo1gActn	Cl2Cl4Zo1gActn
R3	DAPI	DAPI	DAPI	DAPI	DAPI
R4	Cl2Cl4Ki67NaK	Cl2Cl4Ki67NaK	Cl2Cl4Ki67NaK	Cl2Cl4Ki67NaK	Cl2Cl4Ki67NaK
		Floated Stained S4Sl2			
SET 4		DAPI			
		Ki67NaK			
SET3 R1	DAPI	DAPI	DAPI	DAPI	DAPI
R2	CD20Cl15OccNaK	CD20Cl15OccNaK	CD20Cl15OccNaK	CD20Cl15OccNaK	CD20Cl15OccNaK
R3	DAPI	DAPI	DAPI	DAPI	DAPI
R4	Cl2CC3NaKEcad	Cl2CC3NaKEcad	Cl2CC3NaKEcad	Cl2CC3NaKEcad	Cl2CC3NaKEcad
Set5					
R1	gActinNHE3SucrasePept1	gActinNHE3SucrasePept1	gActinNHE3SucrasePept1	gActinNHE3SucrasePept1	gActinNHE3SucrasePept1

Moreover, we are performing RNAscope study on our TMA slides for IFN gamma and TNF alfa. This technique was standardized during my stay. The pending work will be completed after we receive the probes.

Publication: This work is expected to give us valuable information on the role of inflammatory cytokines on intestinal tight junction proteins and transport molecules in various groups of small bowel diseases. We shall write the manuscript and publish it with acknowledgement to ICMR-DHR after we finish it.

iii) Proposed utilization of the experience in India:

1. My experience on mouse model of colitis will help me to expand my research on human celiac disease and inflammatory bowel disease further on experimental level.

2. I have started planning working on the complex role of human tight junction proteins and their mechanism in various small and large bowel diseases.

3. My observation from other works going in in Professor Turner's lab on enteroids and cell monolayer will help me to plan my research works on ex-vivo enteroids better.

3. Valuable techniques learned as sequential multiplex immunofluorescence staining will help me to target multiple targets in spoiling less tissue.

4, I learned valuable analyses software's as Image J, Adobe Illustrator, Cell profiler which I already started using and purchased the AI software.

5. At the last, I perspective changed in Professor Turner's lab/ I learned research administration and already implemented periodic research update meetings for research students in my laboratory.

6. I have already started working on setting up a facility for multiplex IF imaging platform in my department and later will set up high resolution microscopy gradually.

Signature of ICMR-IF 15/7/2023

ICMR Sanction No. INDO/FRC/453/S-49/2022-23-IHD, dated 27/2/2022