Review

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Immunohistochemistry as an important tool for exploring the insights of various aspects of gastro-intestinal tract

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Abstract: The concepts in immunology and techniques in histology have come together in a novel way to create a pioneering discipline known as ImmunoHistoChemistry (IHC), to discover new ways in detecting cell and tissue antigens related to amino acids, proteins and infectious agents by using labeled antibodies. These amalgamation techniques are applied in the disciplines of endocrinology, entero-biology, neurobiology, pathology, tumor biology and pharmaceutical research as a diagnostic tool. The simultaneous advancements in the field of imaging techniques further assisted and widened the application of IHC in molecular studies, thereby facilitating the development of novel therapeutic strategies. This paper attempts to discuss the different aspects of gastro-intestinal tract in relation to its cellular diversity, cellular differentiation, physiology and pathology, through the application of IHC methods.

Keywords: cell identification; cellular diversity; gastro-intestinal tract; immunohistochemistry

Abbreviations

Ab:	Antibody
AEC:	3-Amino-9-Ethylcarbazole Chromogen
Ag:	Antigen
AP:	Extra Avidin Peroxidase
AQP:	Aquaporins
AR:	Antigen Retrieval
B:	Biotin
BSA:	Bovine Serum Albumin
CCK:	Cholecystokinin
CNS:	Central Nervous System
DAB:	3, 3'-DiAminoBenzidine
DC:	Dentritic Cell
G cells:	Gastrin cells
G IR:	Gastrin Immuno Reactive cells
Gfi 1:	Growth factor independence 1
GI hormones:	Gastro-Intestinal hormones
GIP:	Gastric Inhibitory Polypeptide
GIT:	Gastro-Intestinal Tract
GLP-1:	Glucagon–Like Peptide-1
GLUTs:	Glucose Transporters
GP2:	GlycoProtein 2
HRP:	HorseRadish Peroxidase
5-HT:	5-HydroxyTryptamine
IHC:	ImmunoHistoChemistry
M cells:	Membranous / Microfold
MØ:	Macrophages
Na GT:	Na^+ dependent Glucose Transporter
Ngn 3:	Neurogenin 3
PAP:	Peroxidase AntiPeroxidase
PBS:	Phosphate Buffer Saline
PYY:	Peptide YY hormone
RT:	Room Temperature
SGLT1:	Sodium-Glucose Linked Transporter 1
ZnT:	Zinc Transporte

1. Introduction

The development of any branch of science is profoundly influenced by the availability of novel techniques. One such technique, immunohistochemistry (IHC), has given a new dimension to the investigative methods in the field of biological studies. IHC is the method of identifying tissue constituents *in situ* through a specific antigen-antibody interaction, tagged by a visual label (Fig. 1).

In this method, the site of the antibody binding domain is identified either by directly labeling the antibody or using a secondary labeling method [1]. It is a qualitative technique that can reveal even a very small quantity of antigen without considering its absolute quantification. The immunohistochemical procedure for the first time was performed by Coons *et al.* in 1941 and 1942, using a fluorescent antibody in the detection of bacteria in a tissue sample [2, 3]. Till the 1960s, fluorescent antibodies were used to detect the number of tissue antigens. However, the interference by tissue auto-fluorescence and fading of the fluorescent label compelled Tubbs *et al.* to pursue new methods [4]. Thereafter, the investigators tried to label antibodies by using metal-storage proteins, such as ferritin, as an electron dense antibody conjugate [5]. In the late 1960s, Nakano and Pierce succeeded in labeling an antibody with horseradish peroxidase (HRP), an inexpensive and readily available enzyme that could be detected by using a permanent substrate 3, 3'-diaminobenzidine (DAB) [6].

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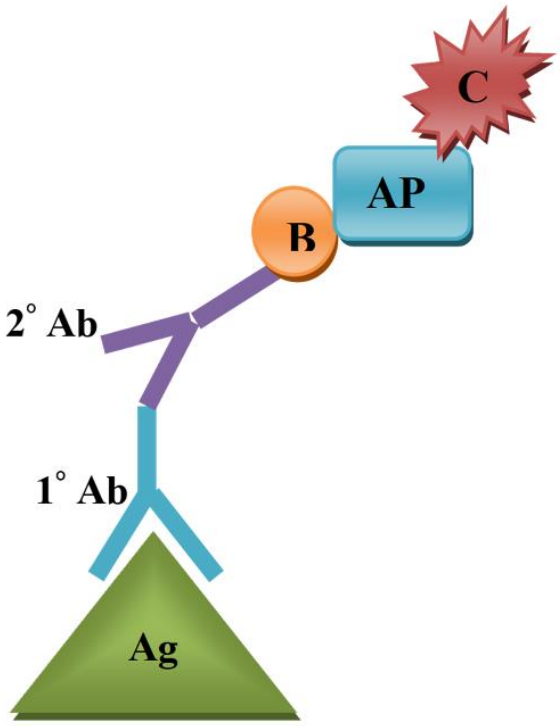

Figure 1: Steps of Immunohistochemistry. **Ag:** Target is antigen (protein targets); **1° Ab:** Primary antibody: Rabbit Polyclonal Antibody; **2° Ab:** Secondary antibody: Biotinylated Goat Anti-Rabbit Immunoglobulins; **B:** Biotin; **AP:** ExtrAvidin Peroxidase; **C:** Chromogen: 3-amino-9-ethylcarbazole (AEC), Colour generated: Reddish brown.

Later, in 1970, Sternberger *et al.* used an enzymatic histochemical method to detect the antigen by the peroxidase antiperoxidase (PAP) labeling method instead of HRP [7]. This PAP-DAB technique became the forerunner of enzyme-labeled methods [8]. In a further expansion of the labeling methods, *e.g.*, colloidal gold, alkaline phosphatase and avidin-biotin complex were used to identify immunohistochemical reactions both at light and electron microscopy levels [9-11]. The wide applications of IHC have made it a versatile tool for molecular studies in endocrinology, gastroenterology, neurobiology, various diagnostic pathologies, pharmaceutical research and cancer research [12]. In 2002, Gown coined the modern term genomic immunohistochemistry due to its application in identifying the presence of genetic mutations and chromosomal alterations [13].

The present article is an attempt to record the application of IHC in understanding the various aspects of the gastro-intestinal tract (GIT), especially the different mucosal cell population (including both endocrine and non-endocrine), cellular morphology and its physiology, gut sensing, brain-gut cross-talk, immunological roles of gut and the gut pathology. As the GIT is the first physiological state of the organism, it plays a vital role in the mechanical and enzymatic processing of the complex and non-diffusible foodstuffs to convert it into the simplest and most diffusible form. This physiological role of GIT provides the basic ingredients, not only for the process of cellular respiration but also for the survival/existence and perpetuation in the higher life forms. Therefore, the study of the different aspects of the gut becomes extremely important. Although both the histological and IHC methods have contributed to a great extent in understanding the gut, only IHC is more advanced and specific to even a very trace amount of antigen.

2. Methodology of IHC

IHC is a very simple and handy experiment that requires minimum infrastructure for its execution. This experiment requires instrumentations such as a simple microtome, incubator, basic glasswares and routine chemicals for processing the histological section, which can be easily procured (Table 1). The histological sections so obtained are subjected to IHC staining protocol (Fig. 2).

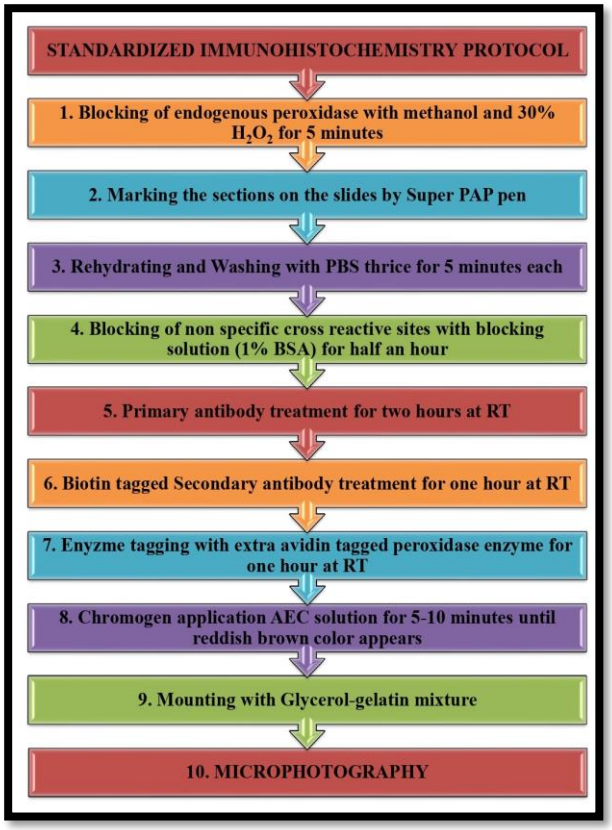

Figure 2: Steps of IHC. At every intermediate step between 5/6, 6/7, 7/8, and 8/9 washing is done with PBS twice each for 5 minutes of duration to remove non-specific bindings. PBS: Phosphate Buffer Saline, RT: Room Temperature.

Kim *et al.*, in a review article, have elucidated the IHC protocols along with their pitfalls and tips [14]. Prior to IHC staining, Antigen Retrieval (AR) step is to be carried out, since in the formaldehyde-based tissue fixation process where the formaldehyde cross-links and masks the epitope of the desired antigen (protein) in the tissue, by forming the methylene bridges [15-18]. The key feature of AR, also called epitope retrieval, relies upon the treatment of sections with heat in an appropriate buffer [19]. Therefore, before executing the IHC steps, it becomes necessary to unmask the epitope and break the cross-links to get better immunoreactivity and reduce the background staining. The IHC staining requires another set of chemicals like primary antibodies (specific to the tissue antigen under investigation), enzyme-labeled second antibody and corresponding chromogenic substrate.

Table 1: List of Instruments, Glassware and Chemicals commonly used in IHC

INSTRUMENTS	LABWARE	CHEMICALS
1. Simple Microtome/ Cryostat Microtome 2. Deep Freezer of -20 °C 3. Motorized Analytical Balance. 4. Digital pH meter 5. Cyclomixer/ Vortex shaker 6. Serological water bath 7. Spreading table 8. Olympus Photomicroscope with a digital camera attachment	1. Super PAP pen liquid blocker 2. Micropipettes 3. Microcentrifuge tube 4. Humidity chamber 5. Glasswares	1. Primary Antibody (Rabbit polyclonal Antibody against tissue Antigen which has to identified) 2. Secondary Antibody conjugate: EXTRA 3 KIT. Biotinylated Goat anti-rabbit IgG+ Avidin peroxidase 3. Chromogen: ACE 101 KIT **For the preparation of the Blocking solution**: 4. Bovine Serum Albumin 5. Triton X100 **For the preparation of Phosphate Buffer Saline**: 6. Sodium dihydrogen phosphate $NaH_2PO_4.2H_2O$ (monobasic) 7. Di-sodium hydrogen phosphate anhydrous AR, Na_2HPO_4 (dibasic) 8. Sodium chloride AR 9. NaOH pellets **For the preparation of Antigen Retrieval Buffer:** 10. Tri-sodium citrate 11. Tween 20 **For the preparation of endogeneous peroxidase blocker:** 12. 30% Hydrogen peroxide 13. Methanol Extra pure **For the preparation of Fixatives:** 14. Picric acid 15. Formaldehyde/Paraformaldehyde 16. Glacial acetic acid **Other Chemicals for obtaining histological tissue sections:** 17. Paraffin wax 18. 2-phenoxy ethanol (a water-soluble anesthesia) 19. Chloroform 20. Egg Albumin 21. Acetone 22. Benzene 23. Xylene 24. Absolute Ethyl alcohol 25. Glycerol 26. Gelatin 27. Sodium azide

The storage of antibodies is most important since it needs a deep freezer (-20 ºC to -80 ºC) with a constant power supply. The beauty of the IHC study is that the antibodies act at low dilution; hence these are required in a very small quantity for the reaction. Therefore, the researchers perform a dilution test in order to determine at which optimum dilution (titer) the primary antibody exhibits the best immunoreactivity [20]. Hofman *et al.*, in their investigation, have given a detailed procedure for determining the optimal staining and highlighted the importance of titrating antibodies for IHC [21]. Another requirement is a photomicroscope with a digital camera attachment for recording the visual color reactions. Therefore, with minimum laboratory facilities, valuable IHC studies can be performed and a wealth of cellular and molecular information can be easily obtained. For the authenticity of any experimental procedure, the control setup is extremely vital; hence in IHC also, the use of proper controls eliminates false positive and false negative results [22]. In performing the control experiment, two different types of procedures can be followed in IHC:

a) Omission control: In this type of control, the omission of either the primary or secondary antibody from the protocol results in no immunoreactions, thereby no observation of any visual color.

b) Pre-adsorption control: This control ensures antibody specificity by demonstrating whether an antibody is binding specifically to the protein of interest or not.

Here the primary antibody solution is pre-incubated with immunizing antigen prior to its application to the tissue section; this results in non-binding with the tissue antigens as the primary antibody's paratopes are already occupied with the immunizing antigen. Therefore, no color development is recorded [23].

In the IHC procedure, the important steps like the application of reagents, incubation and periodic rinsing are carried out manually and carefully, which is tedious work; therefore, the researchers felt a need for automation in IHC to obtain faster results [24]. The current advanced technology provided an automated IHC technique, which guarantees constant, quicker and high-quality labeling in immunostaining steps, but too much dependency on automation, at times, is disadvantageous. Nevertheless, automated IHC has definitely accelerated the IHC research [25, 26].

3. Application of IHC in the identification of different cell types of gastro-enteric epithelium

The entire mucosal epithelium of the alimentary canal comprises several cell types, including both non-endocrine and endocrine cell types. These endocrine cells form only 1% of the total cell population of the enteric epithelium [27]. The non-endocrine cell population includes various enzyme secreting cells, goblet cells, absorptive cells and antigen trapping immune cells or 'M' cells. Each of the differentiated cell types expresses specific/unique 'cell-specific proteins', which can be labeled by IHC (Table 2). Thereby through IHC, all the different cell types, morphology, location, regional distribution and population density have been traced out by different researchers at different times; however, there is still scope to work in this area.

4. IHC studies of gut endocrine cells

GIT is considered the largest endocrine organ, in which its mucosal epithelium consists of a region-specific highly dispersive and diffused distribution of endocrine cells [28]. Each specific gut endocrine cell secretes specific hormones, against which enzyme-labeled antibodies can be generated. The sections of a specific region of the gut are exposed to the enzyme-labeled antibodies, followed by the application of chromogenic substrate, which upon catalysis yields particular color. Positive IHC results reveal a wealth of information regarding the presence, distribution, morphology and physiological status of these endocrine cells. Researchers throughout the world have performed IHC studies of varied gut endocrine cells in different animal models [29-33].

4.1 Morphology of gut endocrine cells

Two major morphotypes of gut endocrine cells, 'Open' type and 'Closed' type, have been reported by Fujita and Kobayashi in 1974 and 1977 [49, 50]. The IHC studies have contributed enormously to a better understanding of the morpho-physiological aspects of both the open and closed type of gut endocrine cells. The open type of gut endocrine cells appears to be a bottleneck shape with a prolonged apical microvillus facing the intestinal lumen. The closed type of cells is of varied shapes, including round, oval, prismatic and amoeboid in appearance.

Table 2: Studies showing the applications of IHC method in identification of different cell types of gastro-enteric epithelium

Cell types in gastro-enteric epithelium	IHC study undertaken: Facts revealed	References
1. Gastro-entero endocrinocytes/Gut endocrine cells	Identification and localization of gut endocrine cells in different animals.	[29-33]
	Studying the morphological features of gut endocrine cells and their classification into 'Open' and 'Closed' types.	[34, 35]
	IHC studies demonstrating the role of gut endocrine cells in sensing the gut luminal contents through the gustducin signaling pathway.	[36]
	Role of gut endocrine cells in endocrine, paracrine and lumenocrine signalling.	[37]
	Neo-role/ Role of GI hormone in extra-gut tissues. IHC revealed that Ghrelin receptors present in the reproductive tract, thus having an additional role in reproduction.	[38]
	Double immune-histochemical staining reveals the co-localization of more than one GI hormone in a single gut endocrine cell, thus suggesting multiple roles of a single cell.	[39-42]
	IHC studies help trace the development and differentiation of gut endocrine cells from stem cells via the notch signaling pathway.	[43]
2. Non-endocrine cells involved in the secretory and absorptive function	IHC methods help to localize Sodium-Glucose Transporters, Aquaporins, Hexose Transporters etc., in these cells.	[44-46]
3. 'M' cells in the inner lining of GIT	IHC studies have revealed many potential markers in human 'M' cells.	[47, 48]

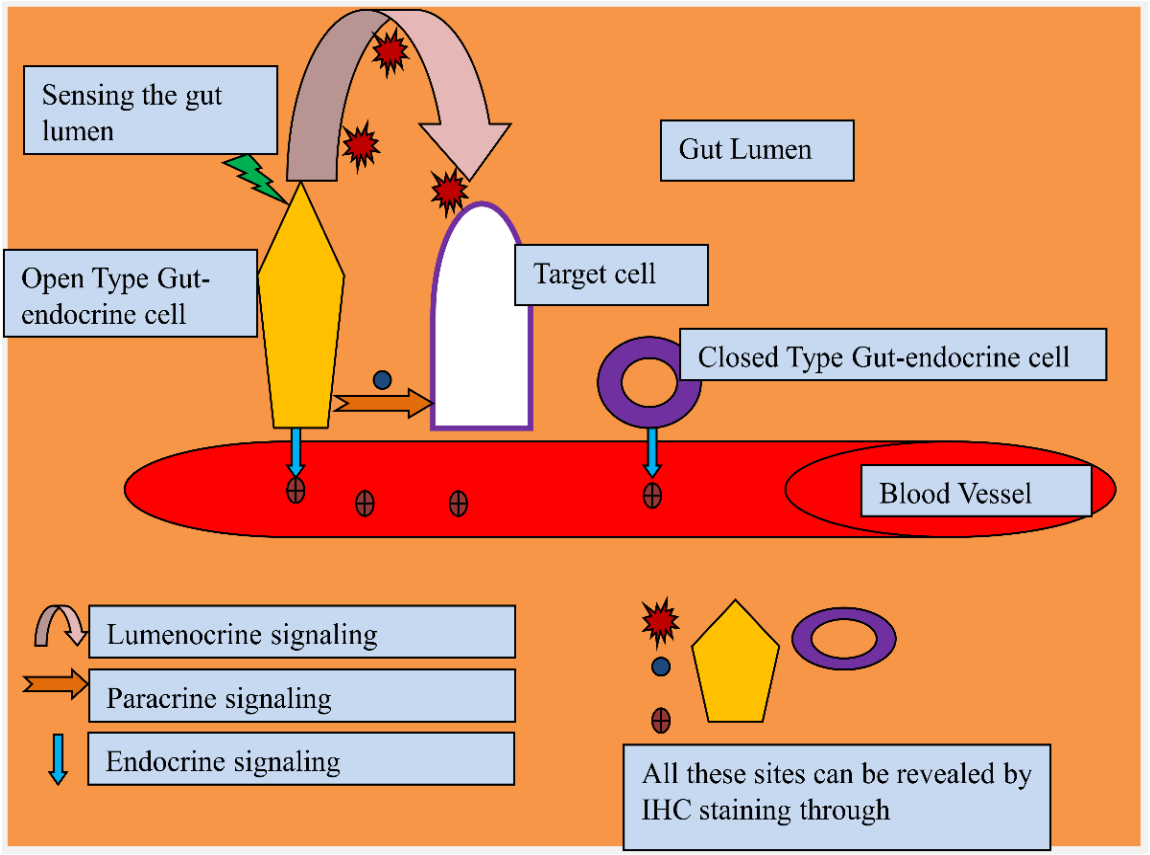

Figure 3: Use of IHC staining in the identification and localization of closed and open type of gut endocrine cells and in tracing the signaling pathway of the gut hormones (hypothetical representation).

These cells are restricted to the basal membrane and do not reach the lumen of the gut and also lack microvilli, and do not come in close contact with the gut lumen [34, 51-53]. Shukla *et al.*, on the basis of the immunohistochemical response, under the light microscope, have identified a third distinct form of Gastrin Immuno Reactive cells (G IR) in the stomach and duodenal regions of *Euphlyctis cyanophlyctis* [35]. The third one has protoplasmic extensions in variable directions, some in the vicinity of gastric glands while others towards the lumen, called a transitional type.

4.2 Regulatory-physiology of gut endocrine cells

4.2.1 Chemo-sensing role of gut endocrine cells

The gut endocrine cells, particularly the open type, have also been involved in the sensing of the gut luminal contents (glucose, amino acids, fatty acids, calcium etc.), through their narrow apical tube extending up to the lumen of the gut. Psichas *et al.*, in their immunohistochemical observations, have reported that upon sensing the gut nutrients, they get stimulated to exhibit hormonal secretory activity at their baso-lateral domain [54].

IHC images of gut endocrine cells reveal the sensory role of the gut epithelium. Sternini *et al.* have described the enteroendocrine cells as the site of 'taste' and 'luminal sensor' in the gastrointestinal chemosensing, as both open and closed type of enteroendocrine cells exhibits α-gustducin receptor immunoreactivity in their apical domain, which is provided with protrusions [27]. It is believed that these cells sense the luminal contents and accordingly respond [36, 55-59].

Endocrine, paracrine and lumenocrine signaling

The so-called gastro-intestinal hormones released by the gut endocrine cells are involved in varied signaling pathways (Fig. 3). The chemical messengers/modulators are not only released into the blood, but they are also released into the lumen of the gut. Therefore, a variety of signal transduction mechanisms can be observed by these molecules and act as a normal traditional hormone. The molecules released in extracellular regions may target the nearby cells in paracrine manner. While others released into the lumen, target the adjacent cells from the luminal surface. IHC studies help in elucidating these signaling pathways. Rao and Shukla have observed luminal gastrin immune-reactivity between the two adjacent villi in the posterior segment of the intestine in a hill-stream fish *Lepidocephalus guntea* during their immunocytochemical study [37]. This case is an example of the lumenocrine pathway of signaling quite commonly observed in the gastro-intestinal hormones.

4.2.2 New roles of GI hormones

IHC is used as a tool for detecting the location and distribution of receptors of GI hormones. Thus, researches have revealed new roles of GI hormones. Ghrelin, a gut hormone involved in the neuroendocrine and metabolic responses to hunger, is now also known to be an important regulator of reproductive functions. Miller *et al.* have provided immunohistochemical evidence for an endocrine

and paracrine role of Ghrelin in the reproductive tissues of sheep [38]. They have localized Ghrelin and its receptors in various tissues of the reproductive tract collected from adult and fetal sheep.

4.2.3 Multiple roles of single enteroendocrine cell

Immunohistochemical analysis in mouse duodenum revealed the co-localization of CCK with secretin, GIP, GLP-1, neurotensin and PYY [39]. Sykaras *et al.* have reported that male mice's duodenal CCK cells express multiple hormones, including Ghrelin [40]. Many enteroendocrine have overlapping roles with the hormones with which they are co-expressed; for example, secretin and 5-HT both promotes bicarbonate release, thus acting synergistically, whereas other co-expressed hormones have antagonistic effects, such as Nesfatin promoting satiety and Ghrelin inducing hunger [41, 42].

Hence, IHC is useful in determining the correlation between two different hormones, although co-localized in a single enteroendocrine cell. In double IHC protocol, immunostaining of one hormone with its antibody, followed by applying a chromogen system that will generate an immune-reaction product, which is reddish-brown in color. This is then followed by the second sequence, in which the second antibody may be used to reveal a different hormone that may be detected using a different chromogen system that will give the blue color. The contrast between reddish-brown and blue color permits the visualization of two different hormones concurrently in the same section. Such a method is referred to as double-staining immunohistochemistry, which is quite useful in co-localisation studies [60, 61].

Role of IHC in studying the differentiation of gut endocrine cells

The entire enteric epithelial cells are periodically renewed. The old cells die out and get replaced by new ones that differentiate from the stem cell population of the enteric epithelium itself. These stem cells differentiate into the endocrine and non-endocrine population of cells [62].

Notch signaling

Notch signaling plays a vital role in this differentiation process [63, 64]. This signaling pathway mediates lateral inhibition between adjacent cells, preventing neighboring cells from adopting the same fate [65]. Therefore, two entero-endocrine cells never present adjacent to each other in the gastro-intestinal tract [66]. Shroyer *et al.*, through double immunostaining analysis, have shown that Gfi 1 expression is restricted to enteroendocrine lineages and also found that the differentiation of gut endocrine cells requires the sequential expression of Math 1, followed by Gfi or Ngn 3 transcription factors [43].

5. IHC study assists in exploring the Gut-Brain axis cross-talk

The relationship between the brain and the gut represents an important area of investigation in neuro-gastroenterology. Enteroendocrine cells play a vital role in gut-brain communication. These cells sense the luminal contents and then release the signaling molecules in the form of hormones, targeting the neighboring cells and neuronal pathways that transmit messages to the brain [67]. Thus, in the brain, there is the constant input of stimuli/signals from the GI tract, which thereafter initiates the appropriate integrated response to the target cells in the GI tract [68]. In support of this, the action of gut hormones, namely CCK, PYY, GLP-1, etc., on vagal afferent neurons is recognized as an early step in controlling nutrient delivery to the intestine by regulating food intake and gastric emptying [69]. In their separate IHC studies, Rehfeld and Dockray have revealed the presence of CCK (a gut hormone) in brain tissues, thus further elucidating the gut-brain connection [70, 71]. Furthermore, Innis and Snyder have demonstrated the presence of CCK receptors in the CNS neurons, which suggests a cross-talk between gut-brain function [72].

6. IHC helps in understanding the functioning of non-endocrine cells of the gastro-intestinal tract

The non-endocrine cells of different gastrointestinal epithelium regions are functionally differentiated cells that perform specific roles related either to the secretory or to the absorptive functions. The membrane characterization of these cells reveals their functional significance. Labeled antibodies were developed against each specific membrane protein used in IHC for tracing the functionally diverse gastro-intestinal cells [44-46, 73-75].

6.1 Na^+ Glucose Transporter

Takata *et al.* had performed Immunohistochemical localization of Na^+ dependent glucose transporter (Na GT) in rat jejunum [44]. Their studies revealed high Na GT positive staining reactions in the apical domain of the intestinal absorptive cells and little Na GT positive staining at the baso-lateral domain. While goblet cells of intestinal epithelium recorded negative Na GT staining.

6.2 Aquaporins and sodium transporters

Zhang *et al.*, through the IHC staining methods, recorded a high-level localization of aquaporins (AQP1, AQP3 & AQP4), epithelium sodium channel and Na^+-K^+ ATPase in the colon region of desert hare, *Lepus yarkandensis* [45]. These results indicate the highest amount of water reabsorption and fecal dehydration, which are the means of adaptation in xeric conditions. Thus, IHC studies are helpful in elucidating the role of membrane aquaporins and sodium transporters of the gut in xeric adaptations.

6.3 Hexose Transporters

Like those of different types of GLUTs in the absorptive cells of the gastro-intestinal tract, hexose transporters play a vital role in the absorption of dietary monosaccharides across the intestinal epithelium. Merigo *et al.*, through their immunohistochemical studies, have established expression of GLUT2, SGLT1, and GLUT5 in short epithelial portions of the large intestinal mucosa of humans [46].

6.4 Zinc Transporters

Zinc is an essential trace element functioning as a cofactor in many metallo-enzymes and also as a structural element in many proteins, including zinc finger motifs. Therefore, the absorption of this small, hydrophilic and charged metal ion is essential and requires a specific membrane transporter protein for its absorption in the GIT. Yu *et al.*, by using

labeled antibodies in their immunohistochemical studies, have reported the expression pattern and localization of ZnT in the different regions of mouse gastrointestinal epithelium [73].

6.5 Transferrin Receptors

Transferrin receptors present in the apical domain of gut epithelial cells play an important role in the uptake of dietary iron bound to transferrin. Kolachala *et al.* and Anderson *et al.* have demonstrated the presence of transferrin receptors in the rat intestinal epithelium through immunohistochemical methods [74, 75].

7. Immunological functions of the gut epithelium, as revealed by IHC studies

Gut mucosa is one of the first lines of defense against foodborne and waterborne pathogens. The enteric epithelium harbors specialized 'M' Cells ('M' = 'membranous' / 'microfold'). These intestinal mucosal cells exhibit distinctive morphological features. Firstly, the apical domain of its plasma membrane possesses a brush border with short microvilli and reduced expression of brush border associated membrane proteins. Second, the baso-lateral domain contains pockets/indentations, colonized by 'B' and 'T' lymphocytes [76-78]. These cells function as antigen trapping cells, transporting them across the intestinal epithelium through the process of transcytosis and finally exposing them to the lymphocyte population in the pocket region (Fig. 4). 'M' cells are known to differentially express specific genes encoding for transmembrane proteins, which act as capture receptors for trapping the antigens like claudin-4, glycoprotein 2 etc. These specific proteins are identified through immunohistochemical methods [79].

Wong *et al.* have carried out an immunohistochemical study of 'M' cells and reviewed the potential markers in their identification and physiological roles [47]. Renfeng *et al.* have used labeled antibodies against one of the potential markers (cytokeratin 18) of 'M' cells to perform immunohistochemical studies for the identification and morphological characterization of these cells in newborn piglets [48]. They compared these results with that of adults and gave information about the age-related development and growth of 'M' cells.

8. Application of IHC studies in the study of Gut pathology

The life of every individual is subjected to disease conditions at one or the other time. The pathological changes brought about in the diseased state widely include inflammatory (due to bacterial and viral infection), metaplastic/tumors, autoimmune, metabolic, degenerative, developmental and genetic impairment as well [80-82].

These pathological changes can be associated with any of the organ systems. During diseased conditions, the biological tissues express different proteins as compared to their healthy counterparts. These differently expressed proteins act as biomarkers, against which antibodies are produced. These antibodies can be used in the diagnostic IHC studies, which reveal vast molecular information about the pathological changes. These results help the researchers to explore appropriate and innovative therapeutic means to counter the pathological conditions. IHC plays an increasingly important role in gastrointestinal pathology practice, particularly in neoplastic disorders; it is used to confirm the diagnosis, identify prognostic/predictive features, and screen for an underlying genetic syndrome [83]. There is an immense contribution of IHC in the field

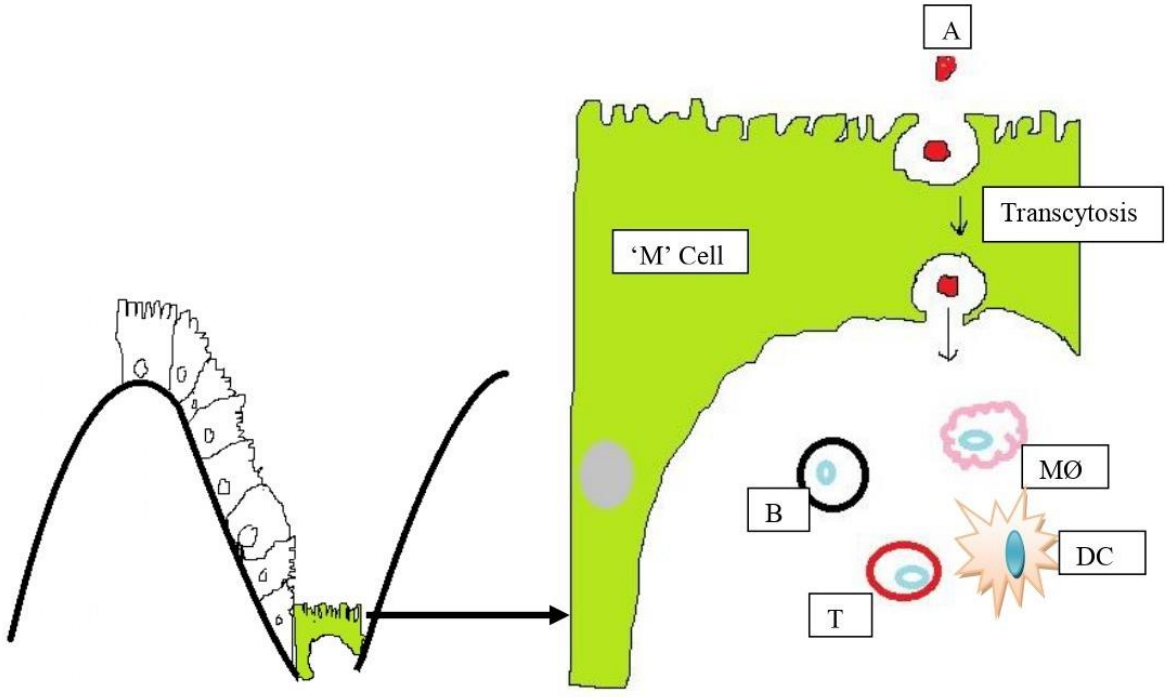

Figure 4: Model representing the localization and functional characterization of 'M' cells in the gut epithelial lining by using the labeled antibodies against potential 'M' cells markers like cytokeratin 18, GP2 and claudin-4 etc. (A= antigen, B= 'B' Lymphocyte, T= 'T' Lymphocytes, MØ= Macrophages, DC= Dentritic cells)

of pathological anatomy, so it is considered the brown revolution of the histopathology laboratory because the most commonly used chromogen DAB yields brown color IHC images [84].

9. Concluding Remarks

The success of IHC is coupled with the advances in imaging tools. The co-evolution of both the techniques brought about a better understanding of the different aspects of Gut Biology; this, in turn, is useful in finding new therapeutic measures in resolving gut-associated pathologies. Designing new nutrient feeds for farm animals and fishes can be made, which will promote more fish growth and farm products to fulfill the ever-increasing demand for a nutritive and cheap source of protein food for the human population.

Declarations

Author Contribution

Both the authors have equally contributed in conceptualizing, literature reviewing, manuscript drafting, revising, and submission. N Venkat Appa Rao has critically evaluated the draft.

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Conflict of Interest

The authors declare that there is no conflict of interest.

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