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Research article

**Association of mesenchymal cells and immunoglobulins with differentiating epithelial cells**

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**Abstract**

**Background:** Mesenchymal-epithelial interactions play an important role in the physiology and pathology of epithelial tissues. Mesenchymal cells either associate with epithelium basement membrane [pericytes and perivascular monocyte-derived cells (MDC)] or reside within epithelium (MDC and T cells). Although intraepithelial mesenchymal cells were suggested to contribute to the epithelium physiology, their association with particular steps in differentiation of epithelial cells, interactions among themselves, and their fate remain unclear. We studied epitopes of mesenchymal cells and their products (immunoglobulins) in stratified epithelium of uterine ectocervix, which is one of the prototypes of complete cellular differentiation from stem into the aged cells.

**Results:** Perivascular CD14 primitive MDC associated with basal (stem) epithelial cells. Thy-1 pericytes of microvasculature secreted intercellular vesicles, which associated with Ki67 postmitotic epithelial cells expressing MHC class I. Intraepithelial T cells showed an association with veiled type MDC [dendritic cell (DC) precursors] among parabasal cells, and exhibited fragmentation after entering intermediate (mature) epithelial layers. Mature DC secreted CD68 and exhibited fragmentation after reaching mid intermediate layers. Binding of IgM was detected at the top of each layer: in the upper parabasal, upper intermediate, and most surface epithelial cells. IgG was confined to the entire superficial layer.

**Conclusions:** These data suggest that the phylogenetically and ontogenetically developed hierarchy of mesenchymal cells (MDC, pericytes, T cells) and immunoglobulins (IgM, IgG) accompanies differentiation of epithelial cells from immature into the mature and aged phenotype. Further studies of an involvement of mesenchymal cells in the regulation of tissue homeostasis may bring novel approaches to the prevention and therapy of tissue dysfunctions characterized by permanent tissue immaturity (muscular dystrophy) or accelerated aging (degenerative diseases).
Background
During last two decades, the contribution of specialized mesenchymal cells, i.e., fibroblast-derived vascular pericytes, monocyte-derived cells (MDC), and lymphocytes, to the proliferation, differentiation, and aging of tissue-specific cells in various epithelial, parenchymal, and muscle tissues has gained increasing interest [1,2,3,4,5,6,7,8,9,10]. Recent developments in the understanding of the role of mesenchymal cells and their products in regulation of proliferation and differentiation of tissue cells were initiated more than seventy years ago, when Alexis Carrel demonstrated that leukocyte extracts, like embryonic tissue extracts, stimulate multiplication of tissue cells were initiated more than seventy years ago, when Alexis Carrel demonstrated that leukocyte extracts, like embryonic tissue extracts, stimulate multiplication of fibroblasts in vitro, and suggested that leukocytes can bring growth-activating substances to tissue-specific cells [11]. Later, in the 1960s and 1970s, lymphocytes were shown to promote tissue growth and regeneration (reviewed in Ref. [12]). In spite of these achievements, our understanding of the interactions between mesenchymal and tissue-specific cells is still in its beginning. While a lot of work has been done on the role of various growth factors and cytokines produced by mesenchymal cells on the cell cycle and death in vitro, and in vivo, the data presented here indicate that phylogenetically and ontogenetically developed hierarchy of mesenchymal cells (MDC, pericytes, T cells) and immunoglobulins (IgM, IgG) accompanies differentiation of epithelial cells from immature into the mature and aged phenotype. Intraepithelial T lymphocytes and mature DC exhibit suicide, and immunoglobulins are associated with aging and apoptosis of epithelial cells.

Results and discussion
Mesenchymal cells
The stratified epithelium of uterine ectocervix consists of four layers of epithelial cells (Fig. 1A). The basal layer (b) is formed by a single row of basal or stem cells. Parabasal layer (pb) is formed by several layers of parabasal (young) epithelial cells. Intermediate layer (im) consists of multiple layers of mature epithelial cells. Superficial layer (s) is formed by several layers of aged cells. These four morphologically distinct layers are divided by three interfaces - b/pb, pb/im, and im/s interface. The parabasal and intermediate layers can be divided into the lower, mid, and upper layers, the superficial layer into the lower and upper layers.

Mesenchymal cells are present in the lamina propria and some invade among epithelial cells. Staining for CD14 (Fig. 1A) shows small primitive MDC in lamina propria but not within the epithelium. Detail, Fig. 1B, shows association of CD14 cells with the basement membrane and extension among basal epithelial cells. Recent study has shown that CD14, a lipopolysaccharide receptor, is itself involved in the stimulation of cell proliferation [21]. Similar association of CD14 primitive MDC with proliferating Ki67 epithelial cells was detected in ovarian cancers (unpublished data). In ectocervix, the staining for Ki67 (inset, Fig. 1A) shows that this marker of proliferation is
expressed in the nuclei of parabasal cells adjacent to the b/pb interface. This indicates that in the stratified epithelium of ectocervix, the Ki-67 is still expressed in the postmitotic cells leaving the basal layer and entering differentiation.

Fig. 1C shows staining for Thy-1 glycoprotein, also called Thy-1 differentiation protein [22], of fibroblasts and pericytes associated with microvasculature (arrows) adjacent to the basement membrane. Detail of Thy-1 staining (Fig. 1D) shows that pericytes secrete intercellular vesicles, which migrate among basal epithelial cells to the b/pb interface, where they collapse into empty structures ("spikes"). Fig. 1E, and detail 1F, show that major histocompatibility complex (MHC) class I molecules are not expressed by basal epithelial cells, but they are strongly expressed throughout the parabasal layer, including lower parabasal cells. Hence, targets for Thy-1 vesicles appear to be parabasal cells adjacent to the b/pb interface,
i.e., epithelial cells expressing MHC class I and entering differentiation.

The intercellular Thy-1 vesicles have been shown by immunoelectron microscopy to exhibit Thy-1 surface expression and to contain a substance lacking Thy-1 staining [23]. They may represent a unique paracrine mechanism, so called "targeted delivery," by which certain growth factor (vesicle content) is delivered by chemotaxis to certain type/stage specific target cells expressing receptor for Thy-1 ligand. However, the receptor for Thy-1 has not been yet identified.

Targeted delivery of some growth factors by intercellular Thy-1 vesicles can be enabled by tissue specificity of Thy-1 glycoprotein carbohydrate moieties [24]. Thy-1 differentiation protein consists of a single Ig domain and represents one of the most primitive and ancestral members of immunoglobulin (Ig) superfamily. The Ig-related molecules have a diversity of functions, but in most cases the common denominator is a recognition at the cell surface [22].

It has been suggested that the involvement of Ig-related molecules in tissue interactions is more primitive than their involvement in the immune system and the immune functions evolved from the sets of molecules mediating tissue interactions [25]. Also, the only function of Thy-1 and other Ig-related molecules is to mediate recognition, with the consequences of recognition being due to the differentiated state of the cells. It requires that the correct ligand and receptor are expressed on the appropriate cells at the right time [25].

MHC class I and class II molecules are other members of Ig superfamily. Fig. 2A shows that large quantities of HLA-DR molecules are secreted by precursors of dendritic cells (DC) among epithelial cells in the mid parabasal layer (arrows). This site-specific HLA-DR secretion is particularly evident when DC precursors are compared with inactive MDC in lamina propria (Ip) or mature DC in intermediate epithelial layers (arrowheads).

T cells expressing CD8, which is another member of Ig superfamily, accumulate in lamina propria, enter epithelium, and migrate through parabasal epithelial layers (arrow. Fig. 2B) expressing MHC class I (Fig. 1E), toward the pb/im interface (dashed line). T cells entering lower intermediate layers exhibit fragmentation (arrowheads). No T cells were detected in the mid intermediate layers or at the epithelium surface.

CD68 epitope of MDC, mucin-like molecule belonging to the lysosomal-associated membrane protein family [26], was expressed by MDC in the lamina propria (Fig. 2C). However, within epithelium, the CD68 appeared during transformation of DC precursors into mature DC, in the upper parabasal layers (arrow). Mature DC (black arrowheads) secreted CD68 among intermediate epithelial cells, and CD68 mucin-like molecules accompanied aging of epithelial cells (white arrowheads) including surface layers.

Staining for CD1a, an Ig-related molecule characteristic for Langerhans’ cells, was not detected in lamina propria (Fig. 2D). Dendritic cell precursors (arrows) and mature DC (black arrowheads) were stained. Mature DC reaching mid intermediate layers exhibited fragmentation (white arrowheads) similar to that of T cells in lower intermediate layers.

Fig. 1A and 2A, C and 2D, show that MDC associated with stratified epithelium of ectocervix exhibit differentiation associated with morphological changes and show changes in immunohistochemical features. Interestingly, although lamina propria of ectocervix showed the presence of primitive (CD14) moderately (HLA-DR) and well differentiated MDC (CD68), the monocyte-derived cells entering epithelium appear to initiate a new differentiation associated with CD1a expression and HLA-DR and CD68 secretion.

Immunoglobulins

Natural autoantibodies are present in the blood of normal healthy individuals, and they are almost exclusively IgM antibodies, although some IgG and IgA natural autoantibodies can also be detected, that bind to a variety of self-antigens, including self IgG [4, 27]. When compared to IgG, the IgM molecules appear earlier in phylogeny and ontogeny [28, 29].

Staining of ectocervical epithelium for IgM is shown in Fig. 2E. Basal and lower parabasal layers were unstained but IgM binding increased toward the pb/im interface (#1, Fig. 2E). In the intermediate layers, similar increase of IgM binding was detected toward the im/s interface (#2). In the superficial layers most prominent staining was found at the epithelium surface (#3). Hence, there was a high affinity of IgM toward the upper cells in the parabasal, intermediate and surface layers (white arrowheads).

The IgG did not bind to the basal, parabasal or intermediate cells, but showed binding to the entire superficial layer (arrowhead, Fig. 2F). These data indicate that natural autoantibodies exhibit a stage-specific binding to epithelial cells. Similar stage-specific binding to epidermis was also detected for natural IgM and IgG autoantibodies in normal human sera [3].
Figure 2
A) Dendritic cell (DC) precursors secrete HLA-DR among parabasal cells (arrows) and differentiate into mature DC (arrowheads). B) T cells migrate through pb layer (arrow) to pb/im interface and show fragmentation after entering im layer (arrowheads). C) Transformation of DC precursors into mature DC at the top of pb layer is associated with CD68 expression (arrow). Mature DC (black arrowheads) secrete CD68 material in im layer accompanying mature and aged epithelial cells (white arrowheads). D) CD1a is expressed by DC precursors (arrows) and mature DC (black arrowheads). Mature DC (Langerhans’ cells) undergo fragmentation in mid im layer (white arrowheads). E) Strong IgM binding (arrowheads) in upper parabasal (1), upper intermediate (2) and upper superficial layers (3). F) IgG binds to the entire s layer. For abbreviations see Fig. 1.
Dual color immunohistochemistry experiments
Basal and parabasal layers of normal ectocervical epithelium show the presence of T cells and MDC. A possibility exists that, beside interaction with epithelial cells, these mesenchymal cell types may interact themselves. Hence, we employed dual color immunohistochemistry for HLA-DR and CD8 epitopes. Fig. 3A shows distribution of HLA-DR MDC (dark field visible light) and Fig. 3B also demonstrates CD8 T cells (dark field fluorescence). It is apparent that MDC interact with epithelial cells adjacent to the basement membrane (open white arrow) and migrate from lamina propria into the epithelium (white arrowhead). Migration of T cells through the basement membrane is also evident (yellow arrowheads).

T cells invading epithelium accumulate in the basal layer, and some of them enter parabasal layer with the assistance of MDC (solid white arrows). Under the pb/im interface (dashed line) T cells are associated with the differentiation of mature DC (solid yellow arrow). The T cells passing pb/im interface exhibit HLA-DR expression (open short yellow arrows). In the lower intermediate layers, the T cells exhibit diminution in size (open long yellow arrow). These data indicate that MDC and T cells continuously migrate into the epithelium, interact

Figure 3
Dual color IHC (HLA-DR peroxidase/CD8 FITC) viewed in dark field visible light (A) and dark field fluorescence (B). A) HLA-DR cells associate with (open white arrow) and migrate through the basement membrane (arrowhead). White arrows indicate DC precursors among parabasal cells, which differentiate into mature DC (yellow arrow) at the pb/im interface (dashed line). Open yellow arrows show activated T cells (see B). B) T cells migrate through (yellow arrowheads) and accumulate above the basement membrane. With the assistance of MDC (white arrows), the T cells enter pb layer and migrate toward pb/im interface. At the interface, they accompany differentiation of DC (solid yellow arrow) and show temporary activation with HLA-DR expression (short open yellow arrows). The T cells entering im layers show diminution in size (long open yellow arrow).
among themselves in the parabasal layer, and show dramatic changes at the pb/im interface.

Fig. 4 shows pb/im interface in detail, with HLA-DR MDC (A), CD8 T cells (B) and both (C). Under interface, the T cells assist differentiation of DC (white arrowheads) and exhibit an unusual elongated shape accompanied by HLA-DR expression (white arrows). Above interface, the mature DC (yellow arrowhead) accompany fragmentation of T cells (yellow arrows). These data indicate that transition of parabasal into intermediate epithelial cells at the pb/im interface is associated with transformation of DC precursors into mature DC with the assistance of activated (HLA-DR) T cells. The T cells entering intermediate layers show a loss of HLA-DR expression and commit suicide with apoptotic fragmentation under the assistance of mature DC.

**Apoptosis-associated proteins in ectocervical epithelium**

In addition to CD8, we also studied changes of CD3 T cell marker. Fig. 5A, and detail in 5B, show fragmentation of T cells (arrowheads) among lower intermediate layers. Fig. 5C demonstrates a lack of T cells at the epithelium surface. In contrast to T cells, fragmentation of mature DC occurred more distantly from the pb/im interface, among mid intermediate layers (Fig. 5D and 5E). No dendritic cells were detected at the epithelium surface (Fig. 5F).

Apoptotic cell death is accompanied by expression of p53 and ras proteins [19, 20]. Arrowheads in Fig. 5G indicate p53 staining of cell fragments at the pb/im interface, and dashed box and Fig. 5H cell fragments among mid layers of intermediate epithelial cells, p53 expression was also detected in epithelial cells undergoing apoptosis at the surface (Fig. 5I). Staining for ras (Fig. 5J,K,L) showed similar features.

Altogether, these observations indicate that intraepithelial T cells and mature DC underwent apoptotic fragmentation among differentiating epithelial cells. Except epithelium surface, no p53 and ras expression was detected in differentiating epithelial cells.

**Complete and restricted mesenchymal-epithelial network**

It is here shown that mesenchymal-epithelial interactions in stratified epithelium of ectocervix exhibit stage-specific interactions between mesenchymal and epithelial cells. They also include interactions of mesenchymal cells themselves either in lamina propria, in association with microvasculature adjacent to the basement membrane, or in epithelium, in association with particular stage of differentiation of epithelial cells.

The stratified epithelium of ectocervix exhibits morphologically distinct stages of differentiation with well defined interfaces, and parabasal, intermediate and superficial layers can be subdivided further (lower, mid and upper layers of parabasal and intermediate cells, and lower and upper layers of surface cells). Hence, the mesenchymal-epithelial interactions should involve epithelial-epithelial interactions between and within epithelial layers. Some of these interactions, like diminution of contact inhibition, can be considered as downstream initiators of tissue regeneration.

We speculate that activation of basal (stem) cells toward proliferation is not a spontaneous process resulting from diminution of contact inhibition, but requires an involvement of so called basic "Tissue Control Unit" (TCU) of the Tissue Control System [23]. The TCU is associated with microvasculature and consists of three elements: perivascular MDC, pericytes, and autonomic innervation. Extensions of MDC among basal epithelial cells (see Fig. 1B) indicate that they may be involved in the recognition of demands of epithelium for regeneration. Perivascular MDC interact with pericytes [30], which are accompanied by autonomic innervation [31]. The innervation is involved in the regulation of tissue quantity, possibly through the regulation of activity of pericytes [23]. From this point of view, one should consider an involvement of epithelial-mesenchymal and mesenchymal-neural interactions as components of mesenchymal-epithelial network.

In the ectocervix, epithelial cells differentiate from immature toward aged cells, which undergo apoptosis with p53 and ras expression (Fig. 5I and 5L). This is accompanied by mesenchymal cells, which interact themselves and undergo differentiation and apoptotic fragmentation within epithelium, and by binding of IgM and IgG. Such situation can be viewed as a "complete mesenchymal-epithelial network" (Fig. 6).

On the other hand, epithelial, parenchymal and muscle cells in many other tissues are somehow prevented from apoptosis, yet most of them do not exhibit infiltration by T cells and apoptosis of mesenchymal cells under physiological conditions. They do, however, contain basic TCUs associated with microvasculature. In addition to tissue regeneration, the TCU is supposed to regulate differentiation of endothelial cells involved in homing of tissue committed T cells and MDC (Fig. 7). The latter have been proposed to carry epigenetically encoded "stop effect" which stimulates differentiation of adult tissues to certain stage, but prevents advanced differentiation and aging (see Ref. [9,10]). Such "restricted mesenchymal-epithelial network" still allows regenera-
Figure 4
Dual color IHC (HLA-DR peroxidase/CD8 FITC) viewed in dark field visible light (A), incident fluorescence (B) and dark field fluorescence (C). A) Interface (dashed line) between pb and im layers. White arrowhead shows differentiating DC, yellow arrowhead shows mature DC. Arrow indicates activated T cell with HLA-DR expression (see below). B) White arrow indicates T cell exhibiting unusual elongated shape at the interface. Yellow arrows indicate residual CD8 expression in fragmented T cell among adjacent im epithelial cells. C) Activated T cell with HLA-DR expression (white arrow) interacts with differentiating DC (white arrowhead). Mature DC (yellow arrowhead) accompany T cell fragmentation (yellow arrows).
Epigenetic programing of the "stop effect" appears to depend on the extent of differentiation of tissue cells during critical period of development, such as termination of the immune adaptation. In simple terms, if the differentiation of tissue cells is suppressed, immature cells will persist during adulthood (muscular dystrophy), and if it is accelerated, premature aging will occur (degenerative diseases) [9].

Conclusions
Our observations indicate that the phylogenetically and ontogenetically developed hierarchy of mesenchymal cells (MDC, pericytes, T cells) and immunoglobulins (IgM, IgG) accompanies differentiation of epithelial cells from immature into the mature and aged phenotype. In agreement with views of others [25], we propose that the complex of interactions involved (Fig. 6) is not uniquely concerned with immunity, but it includes basic interactions which stimulate differentiation of mesenchymal and epithelial cells. Involvement of immune system-related elements (MDC, T cells and B cells) in regulation of epithelium differentiation represents a basic mechanism of immune physiology toward self, from which the immune surveillance toward nonself has evolved. Accordingly, when required, the intraepithelial mesenchymal cells regulating tissue differentiation may be converted into effectors of immunity against infected epithelial cells. Further studies of the mesenchymal-epithelial network may bring novel approaches to the prevention and therapy of tissue dysfunctions characterized by permanent tissue immaturity (muscular dystrophy) or accelerated aging (degenerative diseases).

Materials and methods
Tissues
Fresh samples from uterine ectocervix were obtained from women admitted to the University of Tennessee Medical Center for medically indicated surgical procedures. The specimens were collected from twelve patients who were having a hysterectomy for benign conditions such as fibroids or pelvic pain. Patients with a history of malignancy, endometriosis, or pelvic infections were excluded from the study. All patients gave informed consent prior to their participation in the study, which had been prior approved by the Institutional Review Board.
Figure 6
Presumptive interactions in the "complete mesenchymal-epithelial network" (stratified epithelium of ectocervix). Lower letters (a to p) indicate possible hierarchy of events. Demands for regeneration (a:dashed arrow) result in activation of the basic "Tissue Control Unit" (b-d) associated with microvasculature (Fig. 7), and stimulation of stem cell proliferation by primitive MDC (e; pMDC CD14). Activated pericytes (f; Thy-1 PC), DC precursors (h; DCp HLA-DR), and IgM (i; #1) stimulate gradual differentiation of young epithelial cells. Activated T cells (l; aTC), mature DC (m; DC CD68) and IgM (n;#2) stimulate maturation, and IgG (o) and IgM (p; #3) induce aging of epithelial cells. Dotted arrows (b, d, g, j, k) indicate interactions of mesenchymal cells; AI+, a permissive signal from autonomic innervation. Veiled cells (VC; dashed/dotted arrow) can migrate from the epithelium into lymphoid tissues and modulate production of tissue-committed T cells (TC) and immunoglobulins. BM, basement membrane. b/pb, basal/parabasal; pb/im, parabasal/intermediate; im/s, intermediate/superficial interfaces. For differences with other tissues ("restricted mesenchymal-epithelial network" due to the stop effect of MDC) see Ref. [9, 10].
Figure 7
Schematic drawing of the basic "tissue control unit," which consists of monocyte-derived cells (M), vascular pericytes (P), and autonomic innervation (Al, dashed arrow), and the involvement of other components of the tissue control system (solid arrows). Monocyte-derived cells physically interact with adjacent epithelial (Ep) and endothelial cells (En) through the basement membranes (dotted lines), and influence pericytes, which secrete intercellular vesicles (ICV). These vesicles collapse into the so-called empty spikes (ES) releasing their content (growth factor/cytokine) after reaching target cells. The activity of pericytes is stimulated or inhibited by autonomic innervation (+ or -) which controls quantitative aspects of tissues. Interaction of monocyte-derived cells with endothelial cells may stimulate homing of T lymphocytes (T) and monocyte-derived dendritic cell precursors (DCP; also known as veiled cells) differentiating into mature dendritic cells (DC). The dendritic cell precursors and T cells interact themselves and stimulate advanced differentiation of epithelial cells. IgMs regulate early (IgM\textsuperscript{1}), mid (IgM\textsuperscript{2}), and late differentiation (apoptosis) of epithelial cells (IgM\textsuperscript{3}), and IgG associates with aged cells (see Fig. 6). Dominant role in the regulation of qualitative aspects of tissues belongs to monocyte-derived cell system (including intraepithelial DCP and mature DC), which is supposed to regulate properties of tissue specific cells, including expression of ligands for intraepithelial T cells and autoantibodies. Monocyte-derived cells also carry the "stop effect" information encoded at the termination of immune adaptation, which determines the highest state of epithelial cell differentiation allowed for particular issue. For details see Ref. [9,10].
Peroxidase Immunohistochemistry

Biopsy samples were mounted in 15 × 15 × 5 mm vinyl specimen molds (Tissue-Tek Cryomold biopsy, Miles Inc. Diagnostic Division, Elkhart, IN), embedded in O.C.T. compound (Miles), frozen in liquid nitrogen, and stored in a freezer at -80°C until use. Frozen tissues were sliced into 7 µm serial sections, dried, and stored at 4°C. The immunohistochemical procedure [32] has been done within one week. All steps were performed at room temperature. Universal DAKO LSAB® 2 Peroxidase Kit (DAKO Corporation, Carpinteria, CA), was employed. Briefly, cryostat sections were fixed with acetone, dried, and rinsed in phosphate buffered saline (PBS). Primary antibody, negative control reagent (DAKO), or PBS were applied for 15 minutes. Slides were washed in PBS, and link solution (biotinated anti-mouse immunoglobulin antibody) was applied for 15 minutes. After wash in PBS, slides were incubated with streptavidin-peroxidase conjugate and washed again. Slides were incubated in substrate-chromogen solution, rinsed with PBS, washed in distilled water, counterstained with Harris hematoxylin, dehydrated and mounted.

Primary Antibodies

Primary antibodies were diluted to obtain a final concentration of 5-10 µg/ml of immunoglobulins. The following primary monoclonal antibodies were purchased from DAKO Corporation, Carpinteria, CA: CD4 Langerhans’ cell (clone NA1/34), CD3 T cell (PC3/188A), CD8 suppressor /cytotoxic T cell (DR25), CD14 Monocyte (TUK4), CD68 Macrophage (KP1), HLA-ABC Antigen/ MHC class I (W6/32), IgG (A57H), IgM (R1/69), and Ki67 Proliferating cells (Ki67). Antibodies p53 (PAAb1801) and pan-ras (F132-62) were purchased from Oncogene Science, Cambridge, MA. Thy-1 (F15-42-01) antibody [33] was kindly supplied by Dr. Rosemarie Dalchau. HLA-DR (MEM 12) [34] was kindly supplied by Dr. Ivan Hilgert and Dr. Vaclav Horejsi.

Microscopy and Video Images

Evaluation was performed on a Leitz DM RB (Leica Inc., Wetzlar, Germany) microscope equipped with differential interference contrast and a DEI-470 CCD Video Camera System (Optronics Engineering, Goleta, CA) with detail enhancement. Video images were captured via a CG-7 Frame Grabber (Scion Corporation, Frederick, MD) into the HP Kayak XU800 PC Workstation (Hewlett-Packard Company, Grenoble, France). The captured color images were processed with Scion Image analysis program (Scion Corporation) based on NIH Image software (Wayne Rasband, NIH, Bethesda, MD). To obtain figures, the captured video images were copied into the Microsoft® Power-Point® 97 SR-2 (Microsoft Corporation, Redmont, WA) PC software and size reduced to allow multiple pictures per page. Each figure was assigned with descriptive letters and symbols, and saved in portable network graphic format.

Dual-Color (Peroxidase/Fluorescence) Immunohistochemistry

Dual-color immunohistochemistry [35] experiments were performed by using two unlabeled primary antibodies and the combined peroxidase/fluorescein isothiocyanate (FITC) technique. This was done by visualization of the first unlabeled antibody (HLA-DR) by the peroxidase-conjugated antibody as above [32], without hematoxylin counterstain. The slides were then incubated with the second unlabeled antibody, washed, incubated with FITC-conjugated second antibody, washed again and mounted in aqueous medium [35]. The antibody reagents in the second labeling sequence do not react with those in the first sequence because the latter are altered by the reaction product of diaminobenzidine [36]. Peroxidase-FITC technique allows separate visualization of peroxidase in visible light (either transmitted or dark-field illumination), visualization of FITC alone in incident fluorescence, and combined visualization of peroxidase/FITC in dark-field transmitted fluorescence. Microphotography was performed using color negative films, and negatives scanned to obtain positive images ported into the Power-Point and processed as above.

Abbreviations

MDC, monocyte-derived cells; b, basal layer; pb, parabasal layers; im, intermediate layers; s, superficial layers; Ip, lamina propria; MHC, major histocompatibility complex; Ig, immunoglobulin; DC, dendritic cells; TCU, tissue control unit; PBS, phosphate-buffered saline; FITC, fluorescein-isothiocyanate; IHC, immunohistochemistry.

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