

Macrophage-specific RAM11 monoclonal antibody cross-reacts with basal cells of stratified squamous epithelia

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Abstract: RAM11 is a mouse monoclonal anti-rabbit macrophage antibody recognizing connective tissue and vascular (atheromatous tissue) macrophages. This study demonstrates a cross-reaction of RAM11 with an unknown antigen in rabbit normal epithelial cells. Formalin-fixed, paraffin sections of the New Zealand White rabbit normal skin, oral mucosa, esophagus, small intestine and lung were immunostained with RAM11 antibody followed by goat anti-mouse Cy-3-conjugated antiglobulin. RAM11-positive immunofluorescence was observed in basal layer cells of stratified squamous epithelia (skin, oral mucosa, esophagus). No RAM11 immunostaining was found in any cells of simple (intestinal, bronchial) epithelia. These findings show that basal cells of stratified squamous keratinized and non-keratinized epithelia of the rabbit express an antigenic epitope which is common with that of macrophage antigen recognized by RAM11 monoclonal antibody.

Key words: RAM11 - Macrophages - Stratified epithelium - Keratinocytes - Rabbit

Introduction

RAM11 is a mouse monoclonal IgG1 antibody (mAb) developed by immunization of the mice with the rabbit alveolar macrophage extract. The antibody binds a cytoplasmic, so far uncharacterized antigen. It does not cross-react with human, rat and monkey macrophages [1].

RAM11 has been widely used for immunohistochemical demonstration of rabbit macrophages, particularly in the cellular analysis of the atherosclerotic lesions of cholesterol-fed or heritable hyperlipidemic rabbits [1-4] as well as in morphological analysis of normal rabbit arteries [5]. RAM11 immunoreactivity was also described in macrophages of injured skeletal or cardiac muscle and ocular uvea [6-8].

In the course of experiments on the regeneration of rabbit oral epithelium we observed an unexpected cross-reaction of RAM11 with some epithelial cells. The aim of the present study was to get an insight into that phenomenon.

Materials and methods

Samples of the New Zealand White rabbit tissues (skin, oral mucosa, esophagus, small intestine, and bronchus) collected from six animals were fixed in buffered formalin for 24 h, and routinely embedded in paraffin. Six μ m sections were collected on poly L-lysine-coated glass slides, deparaffinized in xylene, rehydrated in ethanol and washed in phosphate-buffered saline (PBS) (pH=7.4).

For immunofluorescence, after preincubation with 5% normal goat serum for 40 min at room temperature, sections were incubated overnight with mouse monoclonal RAM11 antibodies (Dako, Glostrup, Denmark, code No. M0633), diluted 1:100, in a humid chamber at room temperature. Next, sections were washed extensively in PBS and incubated for 90 min with goat anti-mouse Cy-3-conjugated antibodies (Jackson IR, West Grove, PA, USA, code No. 115-165-146) at a dilution of 1:400. Cell nuclei were counterstained with DAPI (Sigma, Saint Louis, MO, USA). Sections were washed three times in PBS and mounted in glycerol/PBS solution (pH=8.6). Negative controls were performed by omitting the primary monoclonal antibodies during the first incubation.

Sections were examined under Olympus BX50 light/fluorescence microscope. Images were collected using DP-71 digital CCD camera (Olympus, Japan) and IBM PC-class computer equipped with AnalySIS-FIVE[®] (Soft Imaging System GmbH, Münster, Germany) image analysis system.

Results

The immunostaining revealed not only macrophages (Fig. 1), but also RAM11-binding epithelial cells

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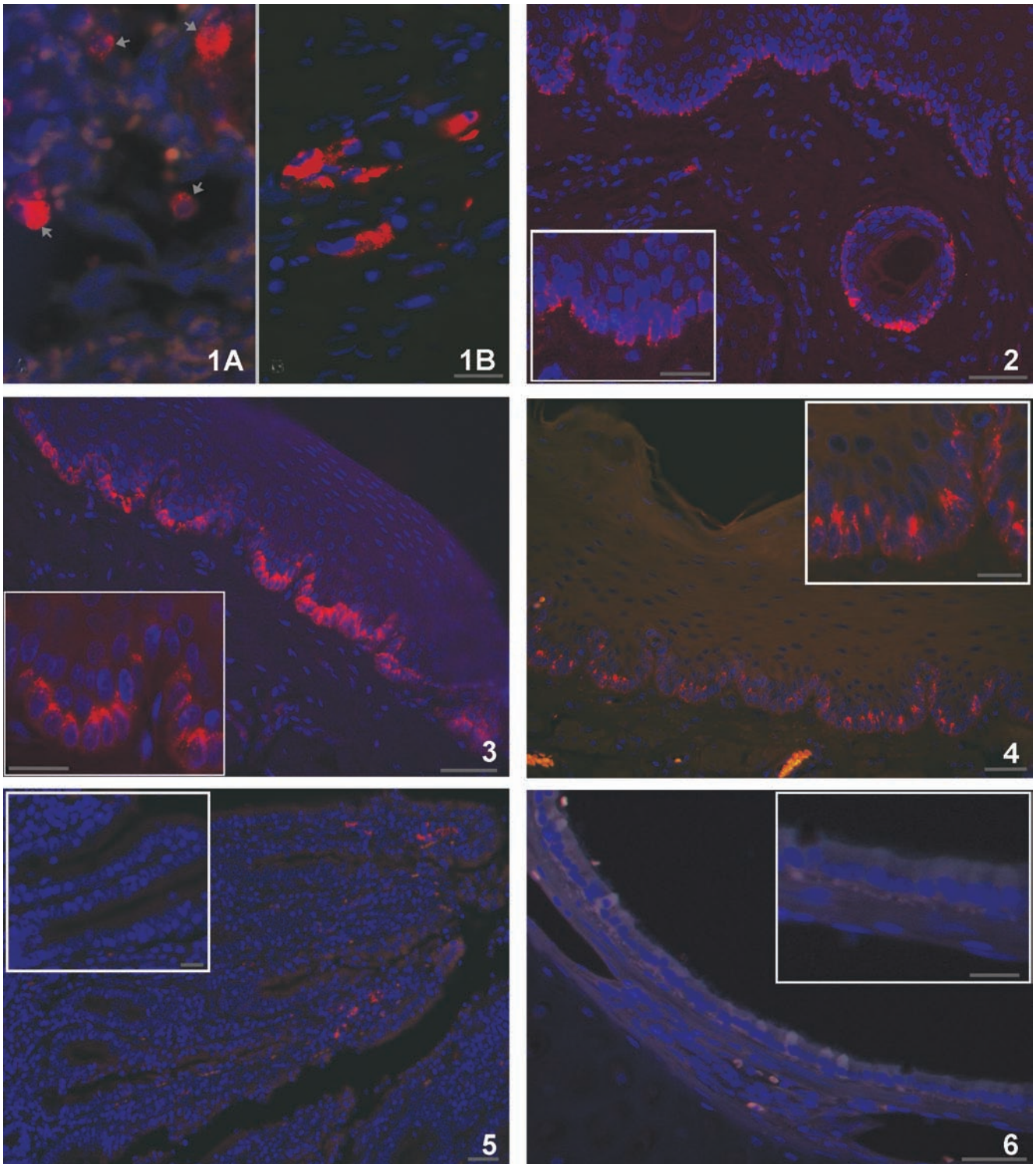


Fig. 1. Alveolar macrophages (**A**) and connective tissue macrophages in the oral lamina propria (**B**) labeled by RAM11 antibody. **Fig. 2.** RAM11-positive immunofluorescence in the basal layer cells of the epidermis and outer root sheath of the hair follicle. **Fig. 3.** RAM11-positive immunofluorescence in the basal layer cells of the oral epithelium. **Fig. 4.** RAM11-positive immunofluorescence in the basal layer cells of the esophageal epithelium. **Fig. 5.** The epithelium covering intestinal villi showing no RAM 11 immunofluorescence. A few fluorescent macrophages can be seen in the connective tissue cores of the villi. **Fig. 6.** Fragment of bronchus showing no RAM11-positive fluorescence in the epithelium. Goblet cells exhibit a weak, nonspecific fluorescence.

Insets in figures 2-6 show the epithelia under higher magnification. Note a granular pattern of immunofluorescence and its preferentially supranuclear/apical location in RAM11-positive cells shown in insets of Figs. 2-4. Bars: in main figures 50 μm , in insets: 20 μm .

located in the basal layer of the stratified squamous epithelia: epidermis as well as oral and esophageal epithelium (Figs. 2-4). Intermediate and superficial epithelial cells were negative in all cases. No RAM11-positive cells were observed in intestinal or bronchial epithelial linings (Figs. 5,6).

In the epidermis and oral epithelium, the intensity of immunofluorescence can be classified as high to moderate, while in the esophagus only as moderate. Generally, almost all cells of the basal layer exhibited immunofluorescence, although considerable intensity differences between the neighboring cells/cell groups could be observed. However, the weakest or negative reaction was always found in cells covering the tips of connective tissue papillae.

In the skin, RAM11 immunostaining was also observed in the basal cell layer of the outer root sheath of hair follicles (Fig. 2). Epithelial cells of mucosal or skin glands were RAM11-negative.

In RAM11-binding epithelial cells, the fluorescence was cytoplasmic, in the form of randomly distributed coarse granules but with a visible tendency to be more intense in the supranuclear/apical regions.

All control samples were negative.

Discussion

This study demonstrates that a macrophage-specific antibody produces an unexpected positive immunostaining of the epithelial cells located in the basal layer of stratified squamous epithelia. The epitope recognized by RAM11 mAb has cytoplasmic localization both in macrophages and epithelial cells.

The cells constituting stratified squamous epithelia undergo a programmed, progressive differentiation from the base where stem cells with high mitotic activity are located to the outermost layers containing finally differentiated cells. The basal (germinal) layer is composed of rapidly dividing low columnar cells resting on the basal lamina. As demonstrated in the epidermis, the basal layer of the stratified squamous epithelia contains two types of proliferating cells: stem cells with high proliferative potential, and so-called transit amplifying cells, which are destined to undergo terminal differentiation after a few rounds of division [9].

We examined the most typical stratified squamous epithelia located in oral cavity and esophagus (non-keratinized) as well as the epidermis (keratinized). In all these epithelia, positive immunostaining was limited only to the basal cells suggesting that the antigen detected is expressed only by the least differentiated epithelial cells. According to Jones *et al.* [9], stem cells identified by bright integrin immunofluorescence are preferentially located at the tips of the dermal papillae. In our experiments, the intensity of RAM11 immunostaining was much lower in these areas, suggesting that

the detected antigen is mainly expressed by the transit amplifying cell subpopulation. This supposition is additionally confirmed by the absence of immunofluorescence in the early foci of epithelial regeneration (unpublished).

One could expect that RAM11 antibody would possibly label antigen-presenting dendritic cells (Langerhans cells) which can be found in all the examined epithelia. Langerhans cells originate from bone marrow, belong to monocyte-derived cells (MDC) and are relatively closely related to macrophages. This is, however, not the case, because Langerhans cells are well known to reside in parabasal and intermediate layers of stratified epithelia and very rarely are observed (during migration) in the basal layer [10]. Interestingly, the absence of RAM11-positive cells in parabasal and intermediate layers indicates that the antibody in fact does not recognize Langerhans cells.

Since the antigen recognized by RAM11 antibody in macrophages has not been characterized so far, it is very difficult to speculate what kind of epitope might be common for macrophages and undifferentiated epithelial cells.

There are several markers of the basal cells in stratified squamous epithelia, including keratin 14 [11] and adhesion molecules such as beta-integrin, E-cadherin, beta-, and gamma-catenins [12]. They are, however, expressed by a variety of other cells in different tissues and by no means can be regarded as specific for macrophages. Another group of basal cell markers are factors involved in proliferation and differentiation, *e.g.* CD90 [13], Tob (Transducer of ErbB2) [14], or Myc-Miz1 complex [15]. Again, these markers are usually not expressed by macrophages, being rather characteristic for various stem/undifferentiated cells.

There is some evidence on mutual interactions between epithelial cells and macrophage family. Data presented by Bukovsky *et al.* [16] indicate that progressive differentiation of MDC toward mature dendritic cells in stratified epithelia remains in association with the particular stages of epithelial cell differentiation. Moreover, MDC are postulated to stimulate basal epithelial cell proliferation.

Macrophage migration inhibitory factor (MIF) is produced by epithelial cells [17-20]. In stratified epithelia (cornea, epidermis), this MIF is located mainly in the cytoplasm of basal layer cells, where it can regulate MDC migration, metabolism and affect their proliferation and differentiation. MIF is also produced by macrophages themselves [21], what makes it a potential candidate for a common antigen of macrophages and epithelia, but on the other hand its synthesis has also been demonstrated in the intestinal epithelium [17] which was found RAM11-negative in this study.

The granular pattern of immunofluorescence observed in the basal layer cells may be indicative of a

secretory character of the detected antigen. In this context, several secretory phospholipase A2 (sPLA2) family members were found to be located only in the basal keratinocytes [22] and some of them are also produced by alveolar macrophages [23]. Again, however, the synthesis of sPLA2 enzymes is not restricted to these two cell types [24] and it has to be clarified whether some of them may be specific only for basal epithelial cells and macrophages.

Taken together, RAM11 recognizes an epitope common for an epithelial and macrophage antigen in the rabbit. On one hand it can not be excluded that two different substances present in basal epithelial cells and macrophages, respectively, share the epitope detected by the antibody, on the other there is a possibility that this unexpected cross-reactivity can indicate some macrophage-basal cell phenotypic and functional association.

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Submitted: 27 February, 2007

Accepted after reviews: 20 April, 2007