Immune Privilege in Hair Growth

Gillian E. Westgate, Robert I. Craggs, and Walter T. Gibson

Personal Products Research Division, Unilever Research, Colworth House, Sharnbrook, Bedford, U.K.

Immunostaining techniques were used to investigate the relationship between immune cells, proteoglycan, and class I MHC distribution in skin during the hair cycle in rats. The growth stage, anagen, was characterized by absence of class I MHC staining on most cells of the lower follicle and presence of chondroitin proteoglycan in the follicle sheath and dermal papilla. Immune cells were few in number and not associated with follicles. Dramatic changes were observed during regression in catagen; class I MHC was expressed on all follicle epithelium, large numbers of activated macrophages aggregated around the follicles, and the chondroitin proteoglycans disappeared from the follicle sheath and dermal papilla. During the resting stage, telogen, class I MHC remained on cells of the secondary germ, but macrophages and chondroitin proteoglycans were absent. These observations lead us to propose a hypothesis of immune privilege in hair growth. J Invest Dermatol 97:417–420, 1991

All hair growth is cyclical with alternating periods of follicle growth (anagen) and rest (telogen) [1]. The transition from growth to rest (catagen) involves a regression, carefully controlled to prevent total follicle destruction. Growing follicles are unusual in that the lower epithelium lacks class I major histocompatibility complex (MHC) antigens [2]. We postulated that the difference in class I MHC antigen expression may provide the basis for the specificity of follicle regression in catagen, and that this process may be driven by cells of the immune system capable of recognizing this difference. The distribution of class I MHC and immunocompetent cells during the hair growth cycle in the rat emphasized the apparent vulnerability of the growing hair follicle and our attention was drawn to a possible role for proteoglycans in masking immunologically vulnerable tissues from immune detection [3]. We therefore expanded our studies to embrace proteoglycan distribution. Due to the wave-like progression of hair growth in the rat [4], skin samples containing follicles in the different stages of the hair growth cycle can be readily obtained. We have studied the first and second hair growth cycles in sections of skin by immunostaining using monoclonal antibodies to chondroitin proteoglycans, class I and class II MHC antigens, and phenotypic cell markers of macrophages and T cells. Our results overall point to the existence of an immunosurveillance system that contributes to the regulation of the hair growth cycle.

MATERIALS AND METHODS

Samples of Wistar rat skin from 17–49 d were snap frozen in thawing isopentane and 5-μm sections cut onto microscope slides coated with poly-L-lysine (Sigma). The sections were air-dried and stored at −20°C until used. Immunostaining was performed with monoclonal antibodies (see Table I) using standard indirect immunoperoxidase staining methods with peroxidase-conjugated rabbit antimouse Ig (DAKO, High Wycombe, U.K.) and diaminobenzidine 0.5 mg/ml (Sigma) with 0.01% H2O2 to develop the substrate. Hematoxylin or methyl green were used as nuclear counterstains. The sections to be treated with antibodies to proteoglycans were pretreated with 0.2 U/ml chondroitinase ABC for 30 min [5,6]. To prevent non-specific staining, all sections were pretreated with 5% rabbit serum; 15% normal rat serum was included in the conjugate dilution. Primary antibodies were used between 1:100 and 1:400 dilution in Tris-buffered saline containing 0.05 M Tris, 0.15 M NaCl, and 1% bovine serum albumin, pH 7.6. The conjugate was used at 1:100 dilution.

Photographs, on Fujicure 400 ASA film, are reproduced at magnification X125 (f), X200 (a–e, g, h, and j–o), and X300 (i).

RESULTS

Anagen During anagen we observed that class I MHC antigens were strongly expressed in the epidermis and follicle epithelium above the level of the sebaceous gland (Fig 1a). However, as is shown in Fig 1b they were only weakly expressed on the outer root sheath cells of the lower, transient, portion of the follicle and undetectable on the cells of the bulb and the cells in the dermal papilla (DP). During this growth period we observed a few cells in the region of the follicle using antibodies to class II MHC (Fig 1c), although this cell staining could be attributed to endothelial cells or pericytes. Cells expressing a macrophage phenotype (OX42 + ve, W3/25 + ve, Table I) were observed throughout the dermis, but no specific association with hair follicles in anagen was noted (not shown). Only very occasionally were T cells observed in the skin, usually in the region of blood vessels (not shown).

Chondroitin 6 sulphate proteoglycan (C6S) was observed both in the DP and in the connective tissue sheath (CTS) in anagen (Fig 1f), with the DP staining much more intensely than the CTS.

Catagen In early catagen, class I MHC distribution was unchanged (not shown), but a striking number of cells expressing class II MHC (Fig 1f) and the macrophage markers OX42 (Fig 1j) and W325 (not shown) were observed in close association with the follicles and in the DP. These are likely to be macrophages rather than Langerhans cells based on their expression of the W3/25
Table I. Source and Specificity of Antibodies Used

<table>
<thead>
<tr>
<th>Code</th>
<th>Ref</th>
<th>Specificity</th>
<th>Working Dilution</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>OX6</td>
<td>[22]</td>
<td>Class II MHC antigens</td>
<td>1:400</td>
<td>Serotec, Oxford, UK</td>
</tr>
<tr>
<td>OX17</td>
<td>[23]</td>
<td>Class II MHC antigens</td>
<td>1:400</td>
<td>Serotec, Oxford, UK</td>
</tr>
<tr>
<td>OX18</td>
<td>[23]</td>
<td>Class I MHC antigens</td>
<td>1:400</td>
<td>Serotec, Oxford, UK</td>
</tr>
<tr>
<td>C65</td>
<td>[5,6]</td>
<td>Chondroitin 6 sulphate proteoglycan</td>
<td>1:100</td>
<td>ICN Biomedicals, Oxford, UK</td>
</tr>
</tbody>
</table>

marker and the observation that W3/25 did not stain dendritic cells in the epidermis.

Changes in C65 distribution were also noted in early catagen, especially in the DP, where the staining was observed to be peripheral in location. This coincided with a condensation of the DP cells, which can be seen in Fig 1m.

In mid-catagen, with active follicle regression and cell resorption, we observed that the remaining epithelial cells now expressed class I MHC antigens (Fig 1f). At this stage activated macrophages were most numerous around the follicle (Fig 1g, j) and some cells were observed within the glassy membrane, invading the epithelial strand (Fig 1g, arrow). Little or no staining for C65 was detectable in either the DP or CTS during this stage, while other structures in the skin, e.g., nerve, remained intensely stained (Fig 1n). T cells were not observed in association with follicles at this stage (not shown).

Telogen In telogen, the end point of the follicle regression, the follicle is a small dormant structure with only the presumptive secondary germ remaining. The cells of the secondary germ expressed class I MHC (Fig 1a); however, it was noted that the DP had remained unstained throughout catagen and telogen. There was no chondroitin proteoglycan staining of follicles (not shown) and macrophages were only associated with the stela, a structure remaining below the follicle containing the remnants of the connective tissue sheath, collapsed vasculature, and nervous system (Fig 1h, k).

Early Anagen Early anagen of the subsequent cycle was associated with re-expression of C65 (Fig 1o). The new follicle bulb cells did not express class I MHC antigens (not shown).

DISCUSSION

The results presented here confirm a previous report [2] that below the level of the sebaceous gland, expression of class I MHC antigens in anagen follicles is virtually absent from some weak staining of the outer root sheath. Thus the growing hair follicle may be

viewed as being immunologically distinct from the infundibulum and epidermis, which are clearly positive for class I MHC antigens.

- Absence of class I MHC antigens on follicle bulb cells may offer a basis for distinguishing between those cells that are resorbed during catagen and those that survive to continue growth in the next cycle. In particular, it may be significant that a population of class I MHC-positive cells was observed in the follicle during catagen and these may contribute to the presumptive secondary germ, which is stained in telogen.

During anagen the extracellular matrix that surrounds and invests the follicle contains C65. In addition, the distribution of unsulphated chondroitin proteoglycan (COS) was investigated using the 1B5 antibody [5] and found to be very similar to that of C65 (not shown here).

However, the results of staining with 1B5 are novel in that previous characterization of this antibody did not demonstrate the presence of COS in skin, but only in cartilage and aorta [5,6]. The increased sensitivity of the immunoperoxidase staining method over immunofluorescence may account for our observations. We believe that these proteoglycans are present mainly in the CTS, rather than the basement membrane of the lower follicle epithelium, on the basis of immuno-electron microscopy [7].

The role of this proteoglycan-rich matrix is not yet established. However, it could conceivably act as a protective screen around the follicle to prevent natural cytotoxic cells recognizing the lack of "self" histocompatibility antigens on bulb cells of the growing follicle. This idea is supported by our observations that during the regression stage, when C65 (and COS) staining is diminished, we have shown increased numbers of "activated" macrophages in and around the follicle. The phenotype of such cells suggests they have a cytotoxic function [8].

These "activated" macrophages seen in catagen could possibly be triggering or driving the follicular regression, or alternatively, they could be responding to some stimulus released by the follicle, a distinct possibility due to the disruption of the connective tissue sheath that takes place during catagen [9]. This process may expose elements of the hair follicle not normally seen by the macrophages and dendritic cells that patrol the skin. Whichever is the case, it is important to note that the increase in cells expressing class II MHC takes place in the absence of activated T cells, normally considered to be the source of class II MHC-inducing interferon [8]. More recently, however, studies have shown that granulocyte/macrophage colony-stimulating factor and tumour necrosis factor both induce expression of class II MHC antigens on tissue macrophages [10,11]. It is an interesting possibility that these mediators are responsible for the increase in class II MHC antigens observed on cells associated with the follicle during catagen.

We believe that these results point to an interplay between class I MHC expression, chondroitin proteoglycans, and macrophages in regulating entry into catagen.

Our understanding of the mechanisms of hair growth regulation is poor, but previous studies have suggested an involvement of immunologic factors. Several hair loss disorders, e.g., alopecia areata [12–14], are immune cell mediated and in addition, hair growth is stimulated by the immunosuppressive agent cyclosporin A [15]. Increased hair growth has also been observed in diseases where an accumulation of glycosaminoglycan is a feature, notably pre-tibial myxoedema and the mucopolysaccharidoses [16–18], and following glycosaminoglycan injections in rabbits [19].

Figure 1. Immunoperoxidase staining of rat skin throughout the stages of the hair growth cycle, using antibodies to class I MHC (OX18) class II MHC (OX6, OX17), macrophage markers (OX42, W3/25), and chondroitin 6 sulphate proteoglycan (3B3). Class I MHC (OX18)—i, epidermis; j, anagen; k, mid-catagen; l, early catagen (OX42); m, mid-catagen (OX17, serial section to c); n, telogen (OX6). Macrophage markers (OX42 and W3/25) —i, early catagen (OX42); j, mid-catagen (W3/25, serial section to c and g); k, telogen (OX42). Note there is non-specific staining of the club hair in j. Chondroitin proteoglycan (3B3) —i, j, k; n, early catagen; o, late catagen; p, early anagen. E, epidermis; F, bulb; N, nerve; CH, club hair.
Our studies on human skin indicate several similarities to the rat
in the distribution of chondroitin proteoglycans [7] and immunocompetent cells* throughout the hair growth cycle. Further studies are planned to investigate these ideas on hair growth regulation to see if they can shed any light on the processes of male pattern baldness.

In conclusion, our results present a unique insight into the regulation of hair growth and substantiate, with new experimental evidence, some long-held beliefs, notably by Billingham [20] and more recently by Paus [21], that the hair follicle is an immune-privileged organ and that hair growth may be under the control of the immune system.

REFERENCES
24. Robinson AP, White TM, Mason DW: Macrophage heterogeneity in the rat as delineated by two monoclonal antibodies, MRC OX41 and MRC OX42, the latter recognising complement receptor type 3. Immunology 57:239–247, 1986
27. Barclay AN: The localisation of populations of lymphocytes defined by monoclonal antibodies in rat lymphoid tissues. Immunology 42:593–600, 1981

* Westgate GE (unpublished observations).