Somatostatin Expression in Human Hair Follicles and Its Potential Role in Immune Privilege

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Immune privilege (IP) is believed to exist in the anagen hair follicle (HF). Studies have shown that downregulation of major histocompatibility complex Class I occurs and immunosuppressive factors are expressed in the HF bulb and bulge. However, demonstration and quantification of functional IP in HF cells are required. We examined the middle (sheath) and lower (bulb) portions of the human HF using quantitative real-time RT-PCR (qPCR), immunohistology, ELISA, *in vitro* coculture with peripheral blood mononuclear cells (PBMCs), and flow cytometry. We found that HF cells, relative to non-follicular epidermal cells, failed to promote allogeneic PBMC proliferation and $CD4^+$ and $CD8^+$ IFNγ production. By qPCR, we found significant downregulation of Class I and Class II HLA alleles in both the bulb and sheath, and upregulation of multiple immunoregulatory genes. It is noteworthy that somatostatin (SST) was significantly upregulated relative to epidermis. By immunohistochemistry, SST was most strongly expressed in the HF outer root sheath, and, by ELISA, cultured sheath cells secreted SST. PBMCs, cultured with stimulatory allogeneic epidermal cells and SST, secreted significantly less IFNγ than controls. Addition of SST antagonists to PBMCs cocultured with allogeneic HF cells increased IFNγ secretion. The data identify SST as a secretory factor potentially contributing to the HF IP repertoire.

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INTRODUCTION

The tissue-protective phenomenon of immune privilege (IP) is believed to exist in the anagen-stage hair follicle (HF) (Paus *et al.*, 2005). First recognition that HFs may exhibit IP was indicated by the studies involving allografts of pigmented skin onto albino skin beds in guinea pigs, in which black hair survived and grew, implying protection of grafted melanocytes by host HF bulbs (Billingham and Silvers, 1971). More recently, scalp HF dermal sheath tissue from a male human was successfully grafted to the arm of a female human (Reynolds *et al.*, 1999). These studies suggest that HFs retain functional IP.

The nature of HF IP has been investigated largely using immunohistological studies. The lower transient cycling portion of anagen-stage HFs exhibit decreased major histocompatibility complex (MHC) Class I expression and low numbers of perifollicular macrophages and T cells during anagen (Westgate *et al.*, 1991; Christoph *et al.*, 2000). Immunosuppressants are secreted from lower HFs, for example, adrenocorticotropin (Slominski *et al.*, 1998), macrophage migration inhibitory factor (MIF) (Meyer *et al.*, 2008), and α-melanocyte stimulating hormone (αMSH) (Botchkarev *et al.*, 1999). HFs may also regulate lymphocyte activity through Fas–Fas ligand as identified in eye graft models (Boffa *et al.*, 1995; Freyschmidt-Paul *et al.*, 2003; Ferguson and Griffith, 2006).

Studies suggest that the HF bulge area and its stem cell reservoir also exhibit IP. By immunohistology, expression of several immunosuppressant factors, such as αMSH, transforming growth factorβ2 (TGFβ2), MIF, and indoleamine 2,3-dioxygenase, have been observed in and around the HF bulge (Meyer *et al.*, 2008). For cell-surface markers, MHC Class I, II, and β-2-microglobulin are decreased, but CD200 is increased in the bulge and adjacent outer root sheath (ORS) relative to non-follicular epithelium (Rosenblum *et al.*, 2004; Ohyama *et al.*, 2006; Meyer *et al.*, 2008). As such, more than one group of HF cells may exhibit IP, although with different characteristics.

It has been hypothesized that breakdown of HF IP may lead to the development of diseases such as alopecia areata and scarring alopecias (Paus *et al.*, 1993; Christoph *et al.*, 2000; Paus *et al.*, 2003). In alopecia areata–affected HFs, MHC Class I and II expression increases (Brocker *et al.*, 1987; Zhang and Oliver, 1994; McElwee *et al.*, 2002). In recent research, an apparent deficiency in Red/IK, an immunoregulatory cytokine,

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Abbreviations: cSST, cyclosomatostatin; HF, hair follicle; IP, immune privilege; MHC I, II, major histocompatibility complex I, II; MIF, migration inhibitory factor; αMSH, α-melanocyte stimulating hormone; ORS, outer root sheath; PBMC, peripheral blood mononuclear cell; qPCR, quantitative real-time RT-PCR; SST, somatostatin; SSTR1, SST receptor 1; TGFβ2, transforming growth factorβ2

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was associated with alopecia areata development in advance of hair loss (Kang *et al.*, 2011).

To date, there have been few quantitative or functional studies to demonstrate that HFs exhibit functional IP. Our aim was to evaluate IP in different portions of healthy human primary anagen HFs, comparing characteristics with normal epidermis as a control. We determined IP-related gene expression in HFs by quantitative real-time RT-PCR (qPCR). The discovery of somatostatin (SST) in HFs led us to cell culture experiments, examining HF cell and SST effects on healthy histoincompatible peripheral blood mononuclear cells (PBMCs). Our research suggests that IP in HFs is mediated partly by SST.

RESULTS

HF cell effect on allogeneic PBMCs

We examined the functional IP capabilities of healthy human HFs *in vitro*. Primary cells from the cycling lower third of the HFs (bulb), the middle third (sheath), or non-follicular epidermal tissues as a control were cocultured with histoincompatible PBMCs, similar to a mixed lymphocyte reaction (Wojtusciszyn *et al.*, 2009; Bocian *et al.*, 2010; Inayat *et al.*, 2010). By detecting IFNγ secretion, we wanted to see whether HF cells attenuated PBMC stimulation. After 5 days, ELISA supernatant analysis revealed that HF bulb and sheath cells elicited significantly less IFNγ secretion from PBMCs than those cultured with epidermal cells (Figure 1a). IFNγ detected in PBMC/epidermal supernatant averaged 35.3 pg ml^{-1}, whereas PBMC/bulb supernatant averaged 18.4 pg ml^{-1} ($P=0.03$) and PBMC/sheath averaged 9.8 pg ml^{-1} ($P=0.007$).

A flow cytometry analysis of IFNγ expression in cell populations on day 5 consistently showed that there were reduced percentages of CD4$^+$IFNγ$^+$ and CD8$^+$IFNγ$^+$ PBMCs in cultures with HF bulb and sheath cells relative to PBMCs cocultured with epidermal cells (Figure 1b and c). When the PBMC/HF percentages were divided by the percentages for PBMC/epidermal cells, there was a significant difference in relative expression between the groups (Figure 1b). Similarly, HF cells did not induce as much T-cell proliferation as epidermal cells with values for CD4$^+$Ki-67$^+$ and CD8$^+$Ki-67$^+$ cells significantly reduced (Figure 1b and c).

IP-related gene expression in HFs

We evaluated mRNA expression levels in 42 IP-related genes in HF bulbs and sheaths relative to non-follicular epidermis by qPCR (Supplementary Table S1 online; Figure 2). As expected, we found multiple class I HLAs A, B, C, and Class II genes *DP*, *DR*, and *DQ*, significantly downregulated in HF bulbs and sheaths compared with the epidermis (Figure 2b). Transporter associated with antigen processing 1 (*TAP1*) was significantly downregulated in HFs. β-2-Microglobulin and MHC Class I

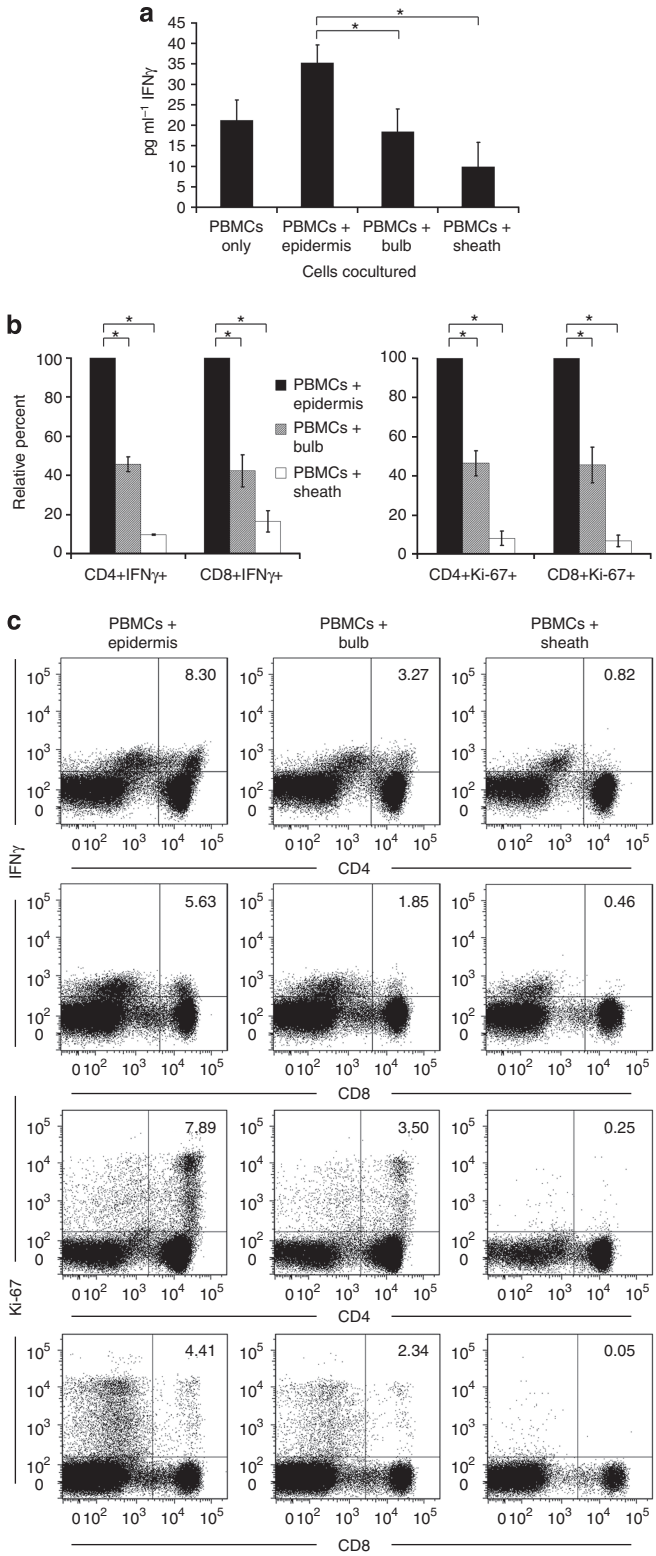

Figure 1. Human peripheral blood mononuclear cells (PBMCs) are significantly less stimulated by allogeneic hair follicle bulb and sheath cells relative to epidermal cells. Non-follicular epidermal cells or hair follicle (HF) cells (2×10^4) were cocultured with allogeneic PBMCs (2×10^5) for 5 days. (**a**) The mean pg ml^{-1} IFNγ detected in supernatant was significantly less in PBMC/HF cultures than in PBMC/epidermal cultures. By flow cytometry, CD4$^+$ and CD8$^+$ PBMCs cultured with HF cells showed significantly less coexpression of (**b**) IFNγ and (**c**) Ki-67 relative to PBMCs cultured with epidermal cells. (**c**) Dot plots show percentage of double-positive cells out of total CD4$^+$ or CD8$^+$ cells; representative of three separate experiments. Bar graphs show the mean of three different experiments ± SEM, with the Student's *t*-test showing significance, $^*P<0.05$.