Influence of Cyclosporin and Prednisolone on RAGE, S100A8/A9, and NFκB Expression in Human Keratinocytes

Squamous cell carcinoma (SCC) is the most common neoplasm among immunosuppressed patients. The immune system plays a prominent role in tumor development. The role of RAGE and its extracellular heterodimeric ligand S100A8/A9 in the development of SCC has recently been suggested. Because this pathway acts through the transcription factor NFκB and thus regulates inflammatory responses, its function may be of particular interest with respect to the development of skin cancer in immunosuppressed patients. Therefore, we aimed to analyze the impact of the most commonly used immunosuppressive agents (cyclosporin A and prednisolone) on RAGE, S100A8/A9, and NFκB expression.

Methods. After study approval from the institutional review board, human keratinocytes were obtained from normal skin of healthy volunteers. For the experiments, the cells were incubated with either cyclosporin A (CsA) (Novartis Pharma, Switzerland) or methylprednisolone (MP) (Sigma Aldrich, Switzerland) at different concentrations for 12 hours. The control cells were treated with vehicles only, ethanol and dimethyl sulfoxide for CsA and MP, respectively. After the incubation period, the cells were homogenized with TRIzol Reagent (Invitrogen) for subsequent messenger RNA (mRNA) extraction and quantitative reverse transcriptase–polymerase chain reaction analysis. Specific primers were used for S100A8 (primer forward, GGGAATTTCCATGCCGTCT; primer reverse, CCGTTCCAGCTGCGACAT), S100A9 (primer forward, CTGTGTGGCTCCTCGGCT; primer reverse, GCCGTTACCTTTTCCAGGAC), RAGE (Hs_AGER_1_SG) (Quantitect Primer assay; Qiagen AG), and NFκB (NF-κB-p65) (primer forward, CCCCCACGAGCTTTGAGAAAG; primer reverse, CAGGGTTCTGGAAACTGTGGAT). Samples were processed in triplicate with 36B4 (primer forward, GCAATGTTGCCAGTGTCTGT; primer reverse, GCCGTTACCTTTTCCAGGAC) as the internal standard. Expression of mRNA was calculated by the ΔΔ threshold cycle. Significance was set at P < .05 (analysis of variance followed by Dunnett post hoc test). All experiments were performed 2 times independently.

Results. Methylprednisolone induced the expression of S100A8, S100A9, NFκB, and RAGE in human primary keratinocytes. Normal human keratinocytes cultured for 12 hours in the presence of different concentrations of MP (ranging from 0.1 mM to 3.0 mM), a potent anti-inflamatory and immunosuppressive drug, significantly increased the expression of the S100A8 and S100A9 mRNA, and the induction was more pronounced for the S100A8 gene (Figure 1). At the concentrations tested, MP also significantly increased the mRNA expression of RAGE and its putative downstream target, NFκB.

Cyclosporin treatment increased mRNA levels of S100A8 and S100A9. Analysis of the influence of CsA on the S100A8/A9-RAGE loop members in vitro demonstrated that this commonly used immunosuppressive drug is an inducer of S100A8 and S100A9 mRNA. Normal human keratinocytes exposed to relatively low concentrations of CsA, ranging from 1 μM to 10 μM, responded by increased expression of the heterodimer S100A8/A9 (Figure 2). Only the highest CsA concentration slightly but significantly decreased the RAGE mRNA level however, most likely reflecting the cytoxic effect of this concentration. Cyclosporin A had no influence on mRNA expression for the RelA subunit of the NFκB complex.

Comment. Multiple clinical phenomena suggest a close relationship between inflammation and SCC development. It has been shown that inflammation is present at low levels in the microenvironment of in situ and invasive SCC of the skin. The difference between this kind of chronic inflammation and acute inflammation lies probably in the orchestration of an effective antitumor immune response. Acute inflammation if strong enough is therapeutically used to treat SCC of the skin, while chronic inflammation can be observed in SCC of the skin where a lack of antitumor defense may allow for tumor formation.

Figure 1. Methylprednisolone (MP) induced increased expression of several genes in cultured normal human keratinocytes. A and B, Real-time polymerase chain reaction analysis of messenger RNA (mRNA) showed up to 5-fold increased S100A8 expression (A) and up to 2-fold S100A9 expression (B) after 12 hours of incubation in different MP concentrations. C and D, In addition, MP upregulated the mRNA levels of RAGE by 3-fold (C) and NFκB up to 14-fold (D) in a dose-dependent manner. The graphs show mean (SD) relative expression. *P < .05; †P < .01; ‡P < .001.
Drug-induced immunosuppression—while profoundly compromising the cytotoxic response of the adaptive immune system, as in organ transplant recipient (OTR) rejection—apparently impairs the inflammatory environment of SCC to a lesser degree. While tumor defense such as a cytotoxic response of the adaptive immune system seems impaired in SCC in OTRs, a persistent inflammatory feed-forward loop via RAGE may contribute to uncontrolled SCC formation in OTRs.

Our results demonstrate that the immunosuppressive agents MP and, to a lesser degree, CsA are able to induce the expression of S100A8A9 in keratinocytes. Promotion of SCC formation through the inflammatory mediators S100A8A9 and RAGE may thus not be dependent on the immune system (inflammatory infiltrate) alone, but keratinocytes in their own right may contribute to the process by the S100A8A9-RAGE feedforward cycle. The influence of immunosuppressive drugs on S100A8A9-RAGE mRNA expression may also suggest a role of these proteins in early-stage SCC development in immunosuppressed patients, facilitating the clinically observed increase of SCCs in these patients.

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Intralesional Immunotherapy With Mycobacterium w Vaccine in Patients With Multiple Cutaneous Warts: Uncontrolled Open Study

Methods. Prior approval was obtained for the study from the institutional review board, and each patient provided written informed consent prior to study entry. Forty patients with multiple cutaneous warts (≥3) were then included in an open-label, interventional study conducted at the Department of Dermatology, Venereology, and Leprology of Sawai ManSingh (S.M.S.) Medical College, Jaipur, India, from December 2010 to August 2011 (8 months). Pregnant women, lactating mothers, children younger than 12 years, patients with a history of hypersensitivity or allergy to vaccines, and those with ulcerated or inflamed warts were excluded from the study.