Molecular Effects of T Lymphocytes on the Regulation of Keratin Gene Expression

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Abstract

The interactions between lymphocytes and the epidermis are very important in autoimmune skin diseases. Mutual interaction between keratinocytes and T cells is effected both by soluble peptides and by direct cell-to-cell contact. We investigated the possibility that direct cell-to-cell contact with T cells may also play a role in the regulation of keratin gene expression. We have transfected human epidermal keratinocytes with the constructs containing promoters of keratin genes and then cocultured them with the HUT78 strain of human T cells. We found that T cells induce transcription of K5, K6, K14 and K16 genes, as well as the RSV viral promoter, but not K17, K10 or the SV40 viral promoter controls.

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Under pathological conditions, keratinocytes become activated, i.e. they become migratory and produce and respond to growth factors and cytokines. Among the markers of keratinocyte activation are keratins K6 and K16, which are produced instead of basal cell-specific keratins K5 and K14 or differentiation-specific keratins K1 and K10. Our previous results [1,2] showed that the growth factors and cytokines directly regulate keratin gene transcription. Therefore, we investigated the possibility that lymphocytes may also regulate transcription of keratin genes. We used the HUT78 line of human T lymphocytes, because this is a permanent cell line and it is constitutively activated. This is the only known T lymphocyte cell line which attaches to keratinocytes that have not been pretreated with interferon γ [3].

Keratinocytes were grown and transfected using our standard protocols [1, 4]. We used an extensive collection of DNA constructs containing keratin gene promoters linked to the CAT reporter gene [1]. After transfection, keratinocytes were grown in the presence or absence of HUT78 cells. We found that the lymphocytes strongly and specifically induce promoters of the K5, K6, K14 and K16 keratin genes but not of K8, K10 and K17 (fig. 1). As a positive control we used ICAM-1 gene promoter, a gift from S.W. Caughman.

Viral promoters also divided into two categories: SV40 promoter was not induced, but RSV promoter was induced (data not shown).

Relatively high numbers of HUT78 cells are required for the effect. Furthermore, both cell types, keratinocytes and lymphocytes, must be in a relatively low passage and at the mid log phase of growth. Difficulties in coordinating the timing and amounts of all reagents makes these experiments extremely difficult to reproduce (S.F. and M.B., data not shown).

In the above experiments, T lymphocytes which grow in suspension, were placed into the culture medium bathing the keratinocytes. Thus, both direct cell-to-cell contact and soluble factors could mediate the effect. To separate the effects of direct cell-to-cell contact from the effects of diffusible mediators, we repeated the experiments but placed the HUT78 cells into insert wells above the keratinocytes. Interestingly, if the cell-to-cell contact is prevented, only

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FOLD INCREASE

FOLD INCREASE

K10

ICAM-1 K17 K10 K5 K14 K6 K16 K8

ICAM-1

In insert wells

T Lymphocytes attached

K14

Keratinocytes normal

K16

Fig. 1. T lymphocytes regulate keratin gene promoters

lymphocytes/ml medium). Keratinocytes were

transfected with DNA constructs containing the

promoters of the keratin genes indicated. Transfected cells were then grown in the presence or absence of HUT78 cells. Note the increase in relative CAT levels due to the HUT78 cells.

Fig. 2. Two modes of regulation by T lymphocytes (attached vs. not attached). HUT78 cells were added either directly onto the keratinocytes or into insert wells. Note that only K16 promoter is induced when direct cell-to-cell contact is prevented.

K6 and K16 genes are induced, which means that these two genes are induced by soluble factors, whereas others require direct contact (fig. 2).

These data indicate that T cells indeed affect gene expression in keratinocytes and that two pathways mediate the effect. One is via soluble factors, the other via direct cell-to-cell interaction.

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