

Correspondence

Thymic stromal lymphopoietin induction by polyinosinic:polycytidylic acid in human keratinocytes is preferentially mediated through protein kinase R and retinoid-inducible gene I and not Toll-like receptor 3

To the Editor:

In a recent report by Kinoshita et al,¹ human keratinocytes have been shown to produce great amounts of thymic stromal lymphopoietin (TSLP) in response to double-stranded RNA (dsRNA), especially in combination with T_H2 cytokines. Because dsRNA and its synthetic analog, polyinosinic:polycytidylic acid (poly I:C), are recognized by at least 4 different receptors in human keratinocytes, namely Toll-like receptor 3 (TLR3), retinoid-inducible gene I (RIG-I), melanoma differentiation antigen (MDA) 5, and protein kinase R (PKR),² we extended these results to the analysis of the responsible receptor mediating TSLP induction. To this end, primary human keratinocytes were cultured and exposed to poly I:C (20 µg/mL; Invivogen, San Diego, Calif), as previously described.² All 4 receptors were blocked with specific inhibitory RNA molecules and pharmacologic inhibitors interacting with downstream signaling of the respective receptors, including bafilomycin (endosomal inhibition of TLR3; Sigma, Deisenhofen, Germany), 2-amino-purin (inhibitor of PKR; Sigma), and SU6668 (inhibitor of the Tank-binding kinase 1 [TBK] common to RIG-I, MDA-5, and TLR3 downstream signaling; kindly provided by GPC Biotech, Munich, Germany), as previously described.² TSLP induction was analyzed by using RT-PCR with primers and probes published by Kato et al³ in the ABI Prism 7000 sequence detection system (Applied Biosystems, Darmstadt, Germany).

These inhibition experiments revealed that TSLP induction by poly I:C was suppressed by PKR- and RIG-I-specific small

interfering RNA (siRNA; between 30% and 50% reduction, depending on the donor) and even more by 2-amino-purin and SU6668 (almost completely in all donors), whereas TLR3- and MDA-5-specific siRNA had no or minimal effects (0% to 20% reduction), and the functional inhibitor of proteasome acidification bafilomycin, which blocks TLR3 activation, had only a marginal effect (10% to 20% reduction, Fig 1).

Thus TSLP induction in human keratinocytes seems to depend on a functional PKR- and RIG-I-TBK signaling axis but not on TLR3 or MDA-5, respectively. This is an interesting observation because the production of *IL8*, a gene that is strongly upregulated in human keratinocytes after poly I:C exposure,⁴ strictly depends on nuclear factor κB (NF-κB). TSLP thus seems to show a pattern of interferon-regulatory factor 3 (IRF-3) activation.³

However, although TSLP in human keratinocytes can be mildly induced by type I interferons, the typical effector cytokines of IRF-3 activation, this protein seems not to belong to the classical type I interferon signature genes, which are upregulated in response to viral infections. In line, the strongest induction of TSLP can be obtained by combining T_H2 cytokines, poly I:C, or both with TNF-α, which is a strong inducer of NF-κB but has no effect on IRF-3.¹

The clinical observation that viral infections tend to deteriorate the skin conditions in patients with atopic eczema might have its correlation by the dsRNA-mediated induction of TSLP and TNF-α through different cell types. Although keratinocytes upregulate TSLP, dendritic cells respond with maturation and the production of TNF-α,⁵ which in individuals prone to a T_H2 immune response with higher production of IL-4 and IL-13, seems to result in a mutual activation of the cells in the skin.

Interestingly, keratinocytes apparently modulate TSLP induction by poly I:C in a different way because human bronchial epithelial cells in which poly I:C induced TSLP upregulation rely

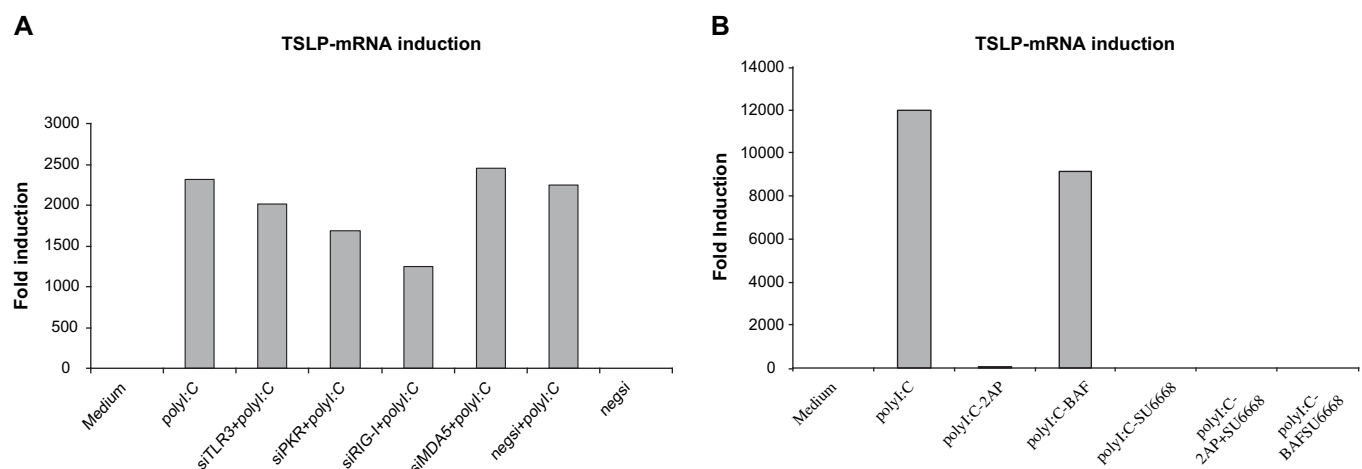


FIG 1. Primary human keratinocytes were incubated with poly I:C (20 µg/mL) for 4 hours. **A**, Real-time PCR was carried out for TSLP, which showed a reduction in the poly I:C-induced transcription, with siRNA targeting RIG-I (30% to 50%), PKR (30% to 50%), and TLR3 (0% to 20%) but not MDA-5 or control siRNA. **B**, When using pharmacologic inhibitors, 2-amino-purin (2-AP; specific for PKR) and SU6668 (specific for the RIG-I, MDA-5, and TLR3 downstream kinase TBK) almost completely (>90%) blocked poly I:C-induced TSLP transcription, whereas bafilomycin (BAF) (an inhibitor of proteasome acidification and thus TLR3 activation) only mildly reduced TSLP induction (10% to 20%). One representative experiment of 3 is shown.

on TLR3, NF- κ B, and IRF-3 but not on RIG-I, MDA-5, and PKR.³ Thus although both cell types respond in a comparable way, possibly interfering strategies aiming at TSLP induction⁶ will require differential approaches.

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