LETTER

A major role for TLR8 in the recognition of vaccinia viral DNA by murine pDC?

The findings of Martinez et al. (1) that vaccinia virus and its DNA are potent inducers of plasmacytoid dendritic cell (pDC)-derived IFN-α in a Toll-like receptor (TLR)9-independent, exclusively TLR8-dependent way came to us as a great surprise (2). The data (1) contradict our results and those of others.

Murine TLR8 gene-transfected cells show a strong induction of an NF-κB luciferease reporter construct after stimulation with CL075 (1). In contrast, others have shown that sole CL075 (also known as 3M-002) was able to trigger murine TLR7 but not murine TLR8-dependent reporter activity (3). This is consistent with our data showing stimulation of human but not murine TLR8 transfectants.

The A-type CpG oligodeoxynucleotides (CpG-ODN) used (1) was fully phosphorothioated, which we found to be biologically inactive. Furthermore, the used concentration of 1 nM is below the range of activity for A-type ODN.

Martinez et al. (1) showed that in vitro flt3 ligand-generated pDC (FL-pDC) produces large amounts of IFN-α in response to CL075, CL087, or CpG-ODN, but the response to CL075 was lost in TLR8-knockdown pDC. In contrast, we found that CL087 and CL075 induced only very little IFN-α in sorted wild-type (WT) FL-pDCs compared with large amounts induced by CpG-ODN or Sendai virus. Furthermore, TLR7-deficient FL-pDCs still produced large amounts of IFN-α, IFN-λ, IL-6, TNF-α, CC chemokine ligand (CCL)3, CCL4, and CCL5 on stimulation with CpG-ODN, but no cytokines were produced in response to Sendai virus, CL075, or CL087.

Data (1) indicate that poly(A) and poly T ODNs were highly stimulatory for IFN-α production in WT but not in TLR8-knockdown pDCs. In our hands, poly A10 and two different poly T ODNs did not induce IFN-α or other cytokines in sorted FL-pDCs or ex vivo-isolated pDCs.

High levels of TLR7, TLR8, and TLR9 transcripts were detected in murine pDCs (1). Our analyses show high-level transcripts for TLR7 and TLR9 but not for TLR8 in murine pDC. In addition, our quantitative proteomic analysis of isolated splenic pDC (4) or sorted FL-pDC revealed the presence of TLR7 and TLR9 but not of TLR8 protein.

A central part of the paper (1) is the observation that the i.v. injection of 10^7 pfu of vaccinia virus strain Western Reserve (VV-WR) induced large amounts of IFN-α in WT but not MyD88-deficient mice 48 h after infection. We and others (5) found that VV-WR is highly inhibitory for the production of IFN-α in vitro and in vivo, and we could not detect any IFN-α in the sera in time kinetics up to 48 h.

In summary, our experiments are consistent with previously published work in the field of vaccinia infection and its recognition, CpG-ODN use, and the role of TLR7, TLR8, and TLR9 for the response of murine pDCs (2–5). However, they largely contradict the results of Martinez et al. (1), which leads us to question the conclusions drawn that VV and VV-DNA are major agonists for murine pDC-expressed TLR8.

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Conflict of interest statement: B.B., H.L., J.P., R.K., P.C., M.S., and H.H. are employees of Bavarian Nordic, B.S. is currently employed by Roche Diagnostic, and S.H. is currently employed by 4SC AG.

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