CD3 HUMANIZED MOUSE MODELS AS VALIDATED TOOLS TO ASSESS IMMUNE-RELATED ADVERSE EVENTS OF T CELL ENGAGERS

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Background

T cell engagers show high efficacy in B cells malignancies. Immune-related adverse events (IrAE), including cytokine release syndrome (CRS), is reported in patients due to on-target off-site effects of T cell engagers (TCE). Translational and predictive assays to assess IrAE of TCE are key to avoid pitfalls in clinical trials. Knock-in humanized mouse models enable the assessment of human-target antibodies in a fully immunocompetent mouse. Indeed, immune responses in CD3 humanized model have been extensively evaluated, and humanization of CD3 has not impaired immune cell distribution. Furthermore, immunization studies showed that T-B cell cooperation is functional in these mice. CD3-TCR complex is also functional, as anti-human CD3 antibodies induce T cell activation. Different classes of TCE showed tumor cell killing ex vivo, as well as tumor growth inhibition in vivo. Here we report the use of CD3 humanized models to assess CRS ex vivo and in vivo.

Methods

Two CD3 humanized mice developed by knock-in were used for ex vivo and in vivo investigation of cytokine production. While one of the models is restricted to one clone of anti-human CD3, the other has been shown to bind several clones of anti-human CD3. Ex vivo, T-cell dependent cellular cytotoxicity assay was performed using different TCE concentrations on splenocytes from CD3 humanized mice. In vivo, CD3 humanized mice were treated with anti-human CD3 and cytokine release in blood, clinical monitoring, body weight and temperature were assessed.

Results

Cytokine release in cytotoxicity assay showed that splenocytes from CD3 humanized mice produced IFN-γ, TNF-α, IL-6, IL-1β, IL-10 and IL-12p70 in a TCE concentration-dependent manner. In vivo, cytokine production was also observed in blood of CD3 humanized mice treated with anti-human CD3. Kinetic of secretion was dependent on the detected cytokine (IFN-γ, TNF-α, IL-6, IL-10) and chemokine (CXCL9, CXCL10, CCL3, CCL2, CCL4). An anti-CD3 dose-effect on cytokine levels of secretion was demonstrated. In parallel, body temperature drop at early timepoints and increase in late timepoints were observed upon treatment with anti-CD3.

Conclusions

Cytokine release and some clinical signs of CRS are reproduced in the two CD3 humanized models described here, validating their value to assess IrAE of TCE. Although the fully functional immune system is one of the main advantages of this model, assessment of mouse biology is a drawback. As a complementary approach, BRGSF-HIS mice, an immunodeficient mouse model reconstituted with human CD34+ cells, showed to reproduce both clinical signs and cytokine production of human TCE-induced CRS.