Mouse Invariant TCR Specific Monoclonal Antibody NKT14: A Novel Tool To Manipulate Invariant NKT Cell Function in Vivo

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Abstract

The iNKT cell represents a novel therapeutic target for important hematologic diseases such as sickle cell disease (SCD) and myeloma. While an antibody specifically targeting human iNKT cells is now in a clinical trial, no surrogate reagent that specifically recognizes murine iNKT cells has been previously reported.

This abstract defines work on a unique, recently developed antibody specifically directed to the T cell receptor of the mouse iNKT cell. These cells are a small subset of T lymphocytes that share characteristics with adaptive as well as innate immune cells. In contrast to conventional T cells they recognize glycolipid antigens presented on the MHC-I-like molecule CD1d. Upon activation they can rapidly release either pro-inflammatory or anti-inflammatory cytokines, depending on stimulus and microenvironment. This enables them to direct downstream immune functions into inflammatory or tolerizing modes. iNKT cell activation has been implicated as a mediator of the chronic inflammation that is found in patients with SCD (Field et al. Blood 121:3321, 2013) suggesting that reduction of activity or iNKT cell depletion may be an effective therapy. The activation of iNKT cells has been shown to have therapeutic effects in multiple hematologic tumors including myeloma, lymphoma, and leukemia (Dhodapkar and Richter Clin.Immunol.140:160, 2011).

Until now, the role of iNKT cells in immune regulation has been studied using iNKT cell deficient inbred mouse strains like CD1d and Jq18 knockout mice or with the iNKT cell activating agent alpha-Galactosylceramide (αGalCer). These tools have weaknesses and limitations. CD1d deficient mice are not only deficient in invariant NK T cells but also other CD1d restricted cells, such as Type 2 NK cells. Jq18 knockout mice have recently been shown to have a substantial decrease in TCR diversity in addition to their iNKT cell deficiency (Bedel et al.,Nat Immuno. 2012 Jul 19;13(8):705-6.). Furthermore, these mouse strains lack iNKT cells from birth and little is known about pharmacologic suppression in iNKT cell competent mouse strains. Although αGalCer can be used to activate iNKT cells in vivo, it induces a persistent iNKT cell anergy after activation. NKT Therapeutics has developed human iNKT cell specific humandized monoclonal antibodies, one of which is currently being evaluated in a Phase I study in patients with sickle cell disease. The human iNKT cell specific antibodies are not cross-reactive to murine iNKT cells.

In order to better understand the potential of pharmacologic modulation of iNKT cell function in pre-clinical disease models, we developed a mouse iNKT specific monoclonal antibody. We have generated both a depleting version (NKT14) and by manipulating the FC-function through mutations we have also generated a non-depleting, activating version (NKT14m). Both are highly specific for mouse iNKT cells and recognize all αGalCer-loaded CD1d tetramer binding cells (Fig. 1A) in multiple inbred mouse strains tested (C57BL/6, BALB/c, NOD, DBA, C3H,NZW, NZW/NZB F1, AKR, SJL and A/J). NKT14m rapidly and very specifically depletes iNKT cells in vivo (Fig. 1B). NKT-14m can activate iNKT cells in vivo and induces release of IFN-Gamma (Fig. 1C).

Conclusion

These novel mouse invariant TCR specific monoclonal antibodies will allow us to better understand the role of iNKT cells in health and disease in order to inform clinical trials of therapeutics which manipulate these unique immune regulatory cells for the treatment of disease.

Figure 1: NKT14 specifically binds and depletes (NKT14) and activates (NKT14m) mouse iNKT Cells

A. C57BL6 splenocytes were stained with NKT14 or isotype control followed by a secondary anti mouse IgG2a monoclonal and αGalCer loaded CD1d Tetramer.
B. C57BL6 mice (n=3 per group) were injected with 50 µg NKT14 or isotype control. Mice were sacrificed 24 hours after dosing and splenocytes prepared, counted and stained for CD3, CD4, CD8 and αGalCer-loaded CD1d Tetramer to assess specificity of iNKT cell depletion.
C. C57BL6 mice were injected with 2µg αGalCer, 50 µg NKT14m or isotype control. 2 hours post dosing mice were sacrificed, splenocytes prepared and stained for CD3 and αGalCer loaded CD1d tetramers to identify iNKT cells, washed, fixed, permeabilized and stained for intracellular IFN-Gamma.

Figure 2: While NKT1T20 blocks CD1d Tetramer binding, NKT4 co-stains with CD1d Tetramer

A. NKT1T20 anti-human ITC mAb blocks CD1d Tetramer binding to Va24Vb11 transfectant
B. NKT4 binds to murine 1.2 NKT Hybridoma and does not interfere with CD1d Tetramer binding

Figure 3: NKT14 depletes iNKT cells in NY1DD mouse model of sickle cell disease

A. Isotype control
B. NKT14m

Figure 4: Antibody Mediated Activation of iNKT Cells Unlike αGalCer Does Not Cause Long Lasting Anergy

A. Isotype control
B. NKT14m

Figure 5: NKT14m Induces Tumor Specific IL-2 Production

A. Isotype control
B. NKT14m

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References

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