**Methods**

**Determination of NKT cell activation in the spleen of the immunized mice**

In order to analyze the status of NKT cells, the spleen was excised and a single cell suspension was prepared as described above. The splenocytes were stained with PE-conjugated α-GalCer-loaded CD1d tetramer and FITC-TCR-β at room temperature in the dark. After an hour of incubation, the splenocytes were washed and fixed in 1% paraformaldehyde. After washing, the cells were suspended in HBSS-BSA and analyzed by using the MACSQuant analyzer 10.

**Secretion of cytokines by splenocytes from the mice after a booster dose**

On day 21 post-immunization, all the five mice were sacrificed and their spleen was taken out. A single cell suspension of splenocytes was prepared and the cells were stimulated with MERS-CoV PLpro (20 µg/ml). After 48 hours, the proliferation of splenocytes was performed as described above. The amount of IFN-γ, IL-4, IL-12 and IL-13 was determined in the culture supernatant.

**Results**

α-GalCer induces the activation of NKT cells in the spleen

Mice immunized with α-GalCer-liposomes had 4.1% splenocytes positive for CD1d/α-GalCer + TCR-β staining as compared to 1.13% splenocytes in the mice immunized with Lip-MERS-CoV PL pro (Suppl.Fig. 1) (P<0.001). Mice immunized with MERS-PLpro-Alum or MERS-PLpro-liposomes had the status of the NKT cells like to that of PBS- or Sham liposomes-injected mice.



**Figure 1.** α-GalCer-MERS-PLpro-liposomes induces the activation of NKT cells in the spleen. The data are represented as the mean ± 95% CI of two independent values.

**A booster dose with α-GalCer-MERS-PLpro-liposomes induced higher secretion of cytokines**

Administration of a booster dose of α-GalCer-MERS-PLpro-liposomes effectively stimulated the splenocytes that secreted significantly greater level of IFN-γ (278 ± 38 pg/ml) as compared to IFN-γ secreted by splenocytes from MERS-PLpro-liposomes (140 ± 24 pg/ml) or MERS-PLpro-Alum (70 ± 17 pg/ml) immunized mice (Suppl. Fig. 2A) (p<0.01 and P<0.001, respectively). However, there was not a significant difference in the levels of IL-4 secreted by the splenocytes from the immunocompetent mice immunized with α-GalCer-MERS-PLpro-liposomes or MERS-PLpro-liposomes or MERS-PLpro-Alum (Suppl. Fig. 2B). A booster dose with α-GalCer-MERS-PLpro-liposomes significantly increased the secretion of IL-12 to 708 ± 56 pg/ml, which was significantly higher as compared to 446 ± 83 pg/ml and 284 ± 28 pg/ml by the splenocytes from MERS-PLpro-liposomes and MERS-PLpro-Alum immunized mice, respectively (Suppl. Fig. 2C) (p<0.001). The splenocytes from α-GalCer-MERS-PLpro-liposomes immunized mice secreted the IL-13 level of 1125 ± 136 pg/ml that was significantly higher as compared to 684 ± 168 and 660 ± 134 pg/ml produced by the splenocytes from the mice immunized with MERS-PLpro-liposomes and MERS-PLpro-Alum, respectively (Suppl. Fig. 2D) (P<0.001).

Interestingly, splenocytes from leukopenic mice secreted IFN-γ level of 175 ± 24 pg/ml that was significantly higher than the IFN-γ levels secreted by the splenocytes from MERS-PLpro-liposomes (105 ± 21 pg/ml) or MERS-PLpro-Alum (39 ± 11 pg/ml) immunized mice (Suppl. Fig. 2E) (p<0.05 and p<0.001, respectively). However, the levels of IL-4 secreted by the splenocytes from the mice immunized with α-GalCer-MERS-PLpro-liposomes or MERS-PLpro-liposomes and MERS-PLpro-Alum were not significantly different (Suppl. Fig. 2F) (p>0.05). The splenocytes from leukopenic mice that received a booster dose of α-GalCer-MERS-PLpro-liposomes secreted the IL-12 level of 456 ± 105 pg/ml as compared to the those of 248 ± 55 pg/ml and 142 ± 32 pg/ml by the splenocytes from MERS-PLpro-liposomes and MERS-PLpro-Alum immunized mice, respectively ((Suppl. Fig. 2G) (p<0.01 and p<0.001, respectively). Similarly, the production of IL-13 was found to be 1275 ± 257 pg/ml, which is greater to IL-13 levels of 757 ± 132 and 697 ± 137 pg/ml secreted by the splenocytes from MERS-PLpro-liposomes and MERS-PLpro-Alum immunized mice, respectively (Suppl. Fig. 2H) (p<0.01).

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**Figure 2.** The levels of IFN-γ, IL-4, IL-12 and IL-13 secreted by the splenocytes from the immunocompetent (A, B, C and D) and leukopenic mice (E, F, G and H) immunized with α-GalCer-MERS-PLpro-liposomes or MERS-PLpro-liposomes or MERS-PLpro-Alum. The data are represented as the mean ± SD of three independent values.