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Immunity analysis kit for canine : a novel tool for the analysis of NK cell activity using whole blood

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Purpose

NK cells perform critical roles in the innate immune response against pathogens and tumors. Functional impairment and a low activity of NK cells were observed in many disease condition including cancer and viral infection. Thus NK cell activity has been suggested a surrogate marker of general immunological functions. In this study, we developed a simple assay to determine canine immunity using a small amount of whole blood.

Materials and Methods

Eighty healthy beagle dogs and twenty seven unhealthy dogs from veterinary hospital were used in this study. All dogs were diagnosed based on histologic and/or biochemical evaluation. NK cell activity was determined using the canine NK activity kit (ATGen).

Keyword : Canine NK cell, Immune potency, IFN-γ, Assay kit

Results

Our assay system was designed on the premise that more potent NK cells secrete higher levels of IFN-γ when activated. Using a proprietary stabilized immunomodulatory cytokine, Promoca, NK cells were stimulated in whole blood. After their activation a quantitative sandwich ELISA was used to determine the levels of IFN- γ. As expected, Promoca induced IFN-γ production and its levels were significantly higher in healthy canine, than in unhealthy ones with severe diseases.

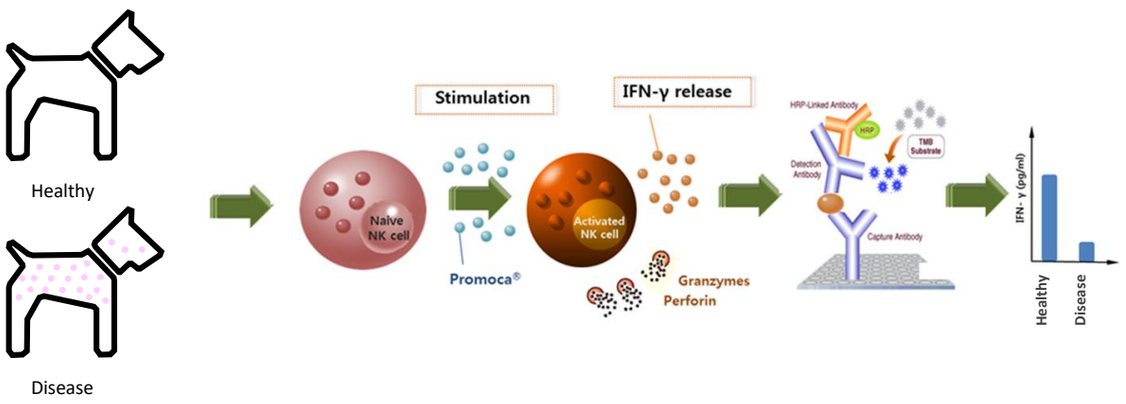

Fig.1. Schematics of NK cell activity measurement using our kit in whole blood.
The freshly collected whole blood from dog is activated by engineered-activator (Promoca). During incubation, NK cells secrete IFN-γ to their full potency. After incubation, the concentration of secreted IFN-γ is measured by ELISA in plasma. This kit can quantify the immune potency of healthy dogs and dogs with immune-mediated diseases by measuring IFN-γ that was secreted by Promoca-activated NK cells in whole blood.

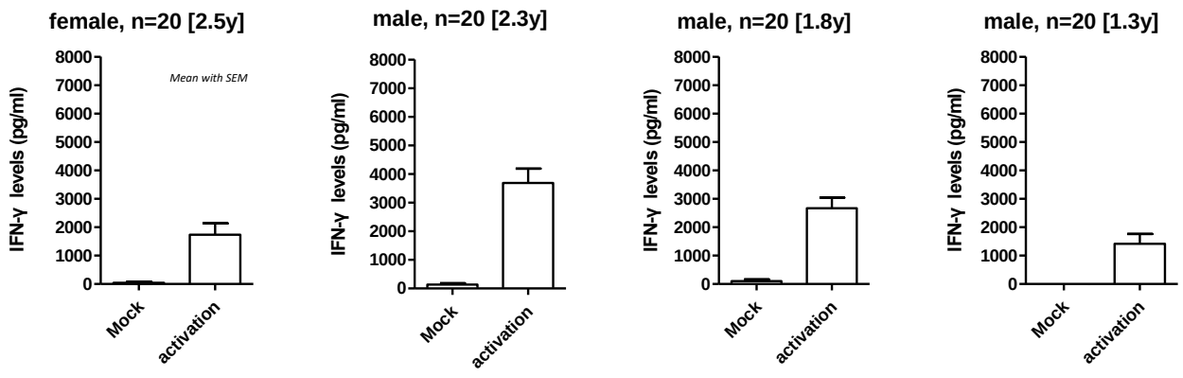

Fig.2. Increased secretion of IFN-γ in Promoca-stimulated dog plasma.
Whole blood was incubated with Promoca for 24 h at 37 ℃. Plasma was obtained by centrifuging (1000x*g*, 15 min), and the concentration of IFN-γ was determined by enzyme-linked immunosorbent assay (ELISA). Induced level of IFN-γ was found in all dogs and considerably higher (2377±2002pg/ml), however non-stimulated sample did not show significant levels of IFN-γ (64±193pg/ml). It appears that Promoca could be utilized as a supportive activator to measure NK cell activity.

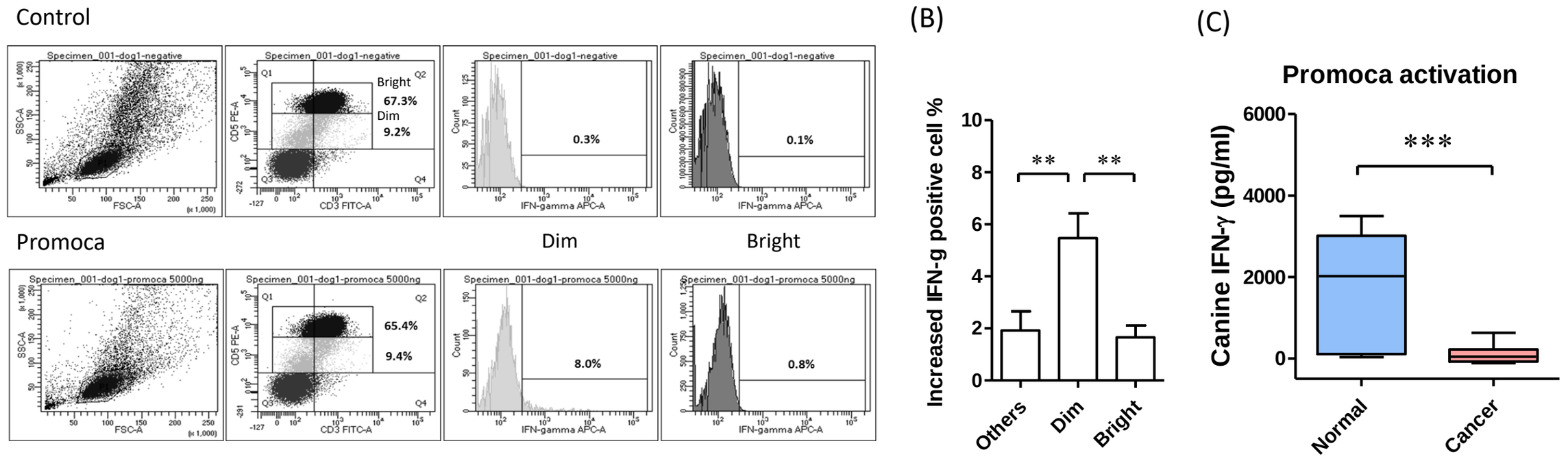

Fig. 3 Determination of major cell type secreting IFN-γ by Promoca-stimulated whole blood.
(A) Whole blood were incubated with Promoca for 16 h then cells were stained with CD3 and CD5. Intracellular IFN-γ staining was done after fixation and permeabilization of cells. (B) The population ratio of cells were average over three healthy dog's flow cytometry results. Induced IFN-γ was found in CD5dim cells by Promoca-stimulation and considerably higher than others. (C) The dogs with cancer showed significantly lower IFN-γ, 112 ± 64 pg/mL compared with healthy subjects, 1823 ± 383 pg/mL. (n=11~13, whiskers; Tukey, ***p<0.001, t-test)

Conclusion

We have developed a high-throughput assay to assess NK cell activity in small amount of whole blood. It is capable of measuring NK cell activity and is simpler than other established methods. This ELISA-based assay could be useful to help in diagnosing and in monitoring diseases associated with functional inhibition of NK cells, such as malignant tumors, viral diseases, and immune-mediated disorders. It will be further validated by clinical studies, especially cancers. This kit should be a good monitoring tool for health condition of pet dogs.

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