# Transfer protocol of human HSC into NOG mice

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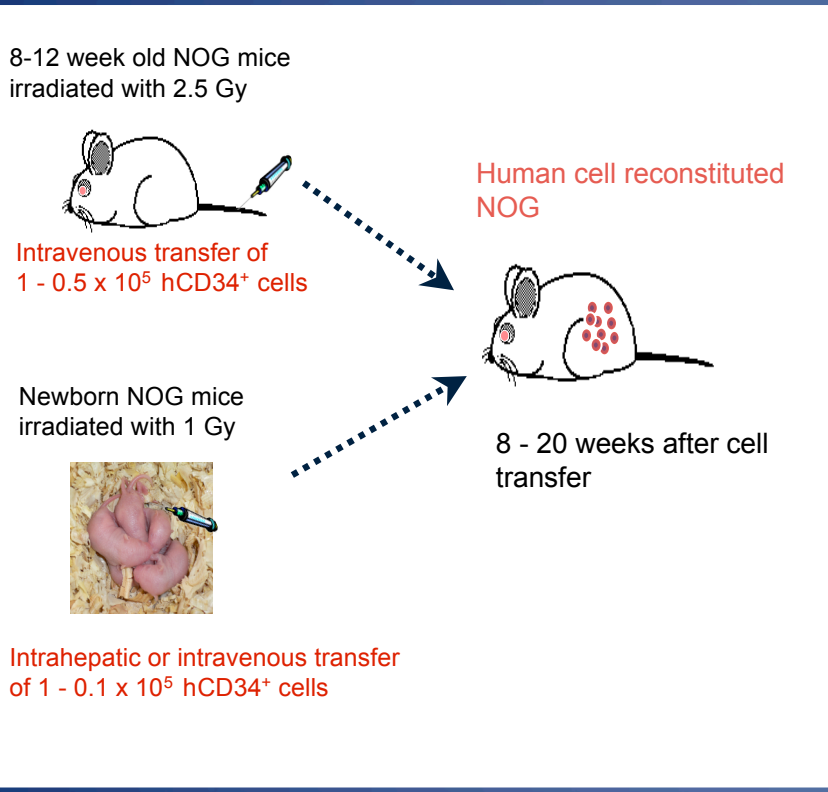


Mice:

Adult NOG mice are aged 8 - 12 weeks. Newborn mice are 1 – 2 days old.

Irradiation:

Irradiation is performed with 2 - 2.5 Gy to adult mice and with 1 Gy to newborns one day prior to cell transfer under SPF conditions. Mice less than 10 g body weight may die with this dose irradiation.

Cell preparation

1. When CD34+ cells (e.g. 1 x $10^6$/tube) are frozen, cells are thawed in a 37℃ water bath.
2. Transfer cells into 50 mL conical tube and add 1 mL of PBS containing 2% fetal bovine serum (2%FBS-PBS) drop by drop shaking the tube slowly.
3. After further addition of 18 mL of 2%FBS-PBS, centrifuge it at 1,200 rpm for 5 min at room temperature.
4. Re-suspend the cells in 10 mL of 2%FBS-PBS, re-centrifuge.
5. Re-suspend in 2 mL of PBS.
6. Using 15 $\mu$L of the suspension, count the cell number and viability after adding 15 $\mu$L of 0.25% trypan-blue solution. Add a further 3 mL of PBS to reach a total of 5 mL.
7. Transfer 1 mL of the cell suspension into each 1.5 mL cryotube.
8. Put the tubes into a 50 mL conical tube and carry it to the animal facility.
9. After dipping it in an antiseptic solution, carry it into the animal room.

Cell transfer

For adult mice

1. 0.25 mL (1 - 0.5 x $10^4$) of the cell suspension is injected into mice via the tail vein with a 1 mL syringe with 27 G or a Microinjector syringe with 29 G under slight anesthesia with Isoflurane.

For newborn mice

1. Slightly anesthetize the newborn with Isoflurane.
2. Hold the newborn with left hand so the head of the mouse face right.
3. 0.1 mL (1-5 x $10^4$ ) of the cell suspension is injected via the face vein with a Microinjector syringe with 29 G.
4. Return the newborn to the mother gently, do not give it a shock.

# Engraftment of human cell in NOG mice

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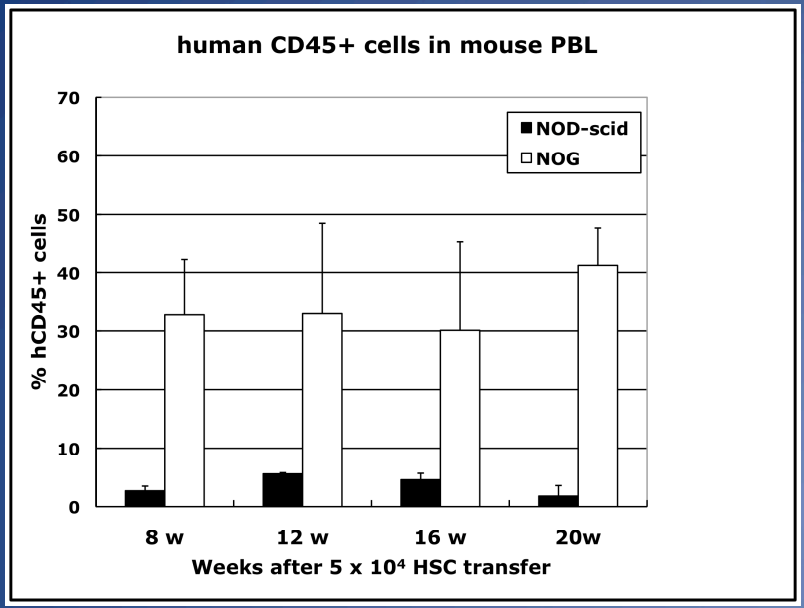


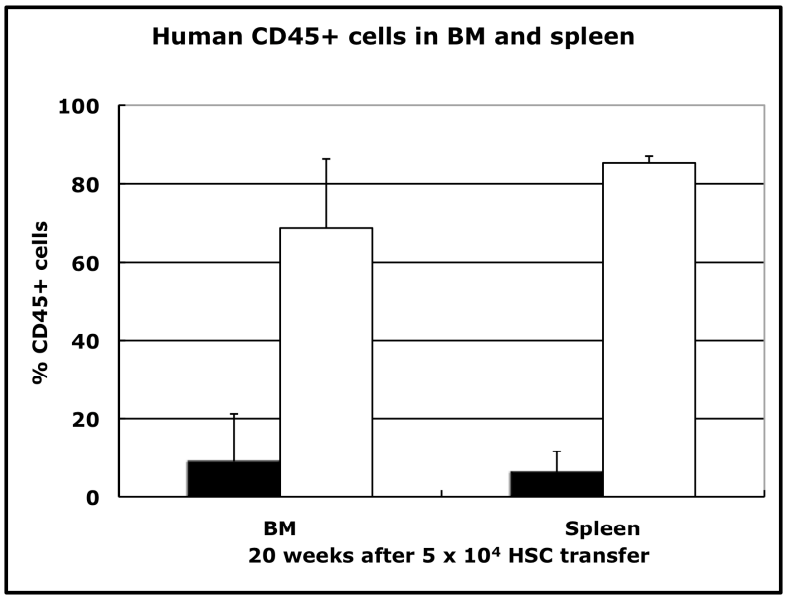


8 - 20 weeks after intravenous transplantation of 5 x $10^4$ human cord blood derived CD34+ cells into NOG and NOD-*scid* mice, human CD45+ hematopoietic cells in peripheral blood of mice were detected in flow cytometry. The high engraftment rate of human cells was observed in NOG mice.

Table 1. Limiting dilution assay in NOG mice.

| CD34+ cell dose | No. mice transplanted | No. mice successfully engrafted* |
|---|---|---|
| 1,000 | 5 | 5 |
| 200 | 3 | 2 |
| 100 | 6 | 3 |

* Successful engraftment was defined the presence of at least 0.1% human CD45+ cells in bone marrow by flow cytometory.

In order to evaluate the frequency of CB $CD34^+$ cells capable of engrafting NOG mice, these mice were transplanted with CB $CD34^+$ cells in a limiting dose. All recipient mice transplanted with 1 x $10^3$ CB $CD34^+$ cells showed successful engraftment (>0.1%). Further, as few as 100 cells could be engrafted in 2 of 6 mice and their multi-lineage differentiation.

# Multi-lineage cell differentiation from HSCs in NOG mice

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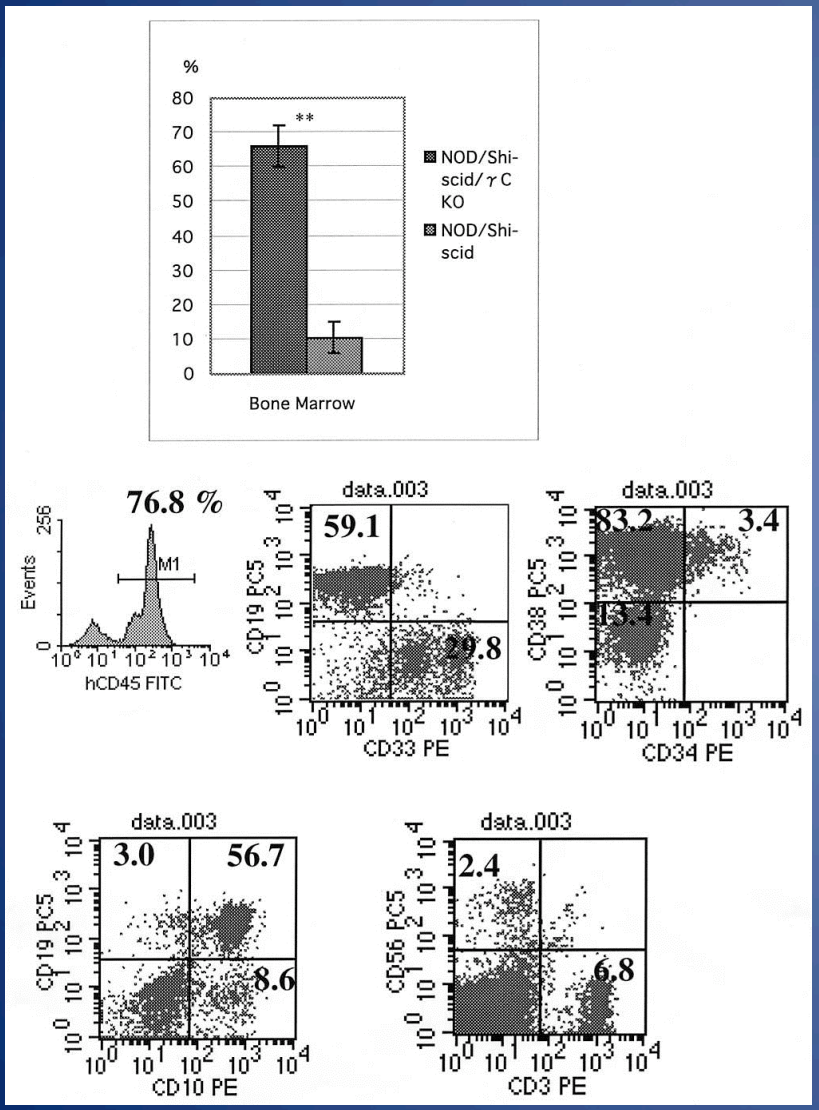
## In bone marrow




The rate of human CD45+ cells in bone marrow of transplanted mice and the high efficacy of multi-lineage cell differentiation. 11 weeks after CD34+ cell transplantation, bone marrow were removed from NOD/SCID/$\gamma_c^{null}$ and NOD/Shi-*scid* mice treated with anti-asialo GM1 antibody two days before transplantation, and subjected to flow cytometry. NOD/SCID/$\gamma_c^{null}$ showed significantly higher percentage of CD45+ cells. Multi-lineage cells have been differentiated from CD34+ cells with high efficacy (* vs **: $P<0.01$).


1. Ito, M., H. Hiramatsu, K. Kobayashi, K. Suzue, M. Kawahata, K. Hioki, Y. Ueyama, Y. Koyanagi, K. Sugamura, K. Tsuji, T. Heike, and T. Nakahata. 2002. NOD/SCID/gamma(c)(null) mouse: an excellent recipient mouse model for engraftment of human cells. *Blood* 100:3175-3182.
2. Yahata, T., K. Ando, Y. Nakamura, Y. Ueyama, K. Shimamura, N. Tamaoki, S. Kato, and T. Hotta. 2002. Functional human T lymphocyte development from cord blood CD34+ cells in nonobese diabetic/Shi-scid, IL-2 receptor gamma null mice. *J Immunol* 169:204-209.
3. Saito, Y., Y. Kametani, K. Hozumi, N. Mochida, K. Ando, M. Ito, T. Nomura, Y. Tokuda, H. Makuuchi, T. Tajima, and S. Habu. 2002. The in vivo development of human T cells from CD34(+) cells in the murine thymic environment. *Int Immunol* 14:1113-1124.
4. Hiramatsu, H., R. Nishikomori, T. Heike, M. Ito, K. Kobayashi, K. Katamura, and T. Nakahata. 2003. Complete reconstitution of human lymphocytes from cord blood CD34+ cells using the NOD/SCID/gammacnull mice model. *Blood* 102:873-880.
5. Watanabe, Y., T. Takahashi, A. Okajima, M. Shiokawa, N. Ishii, I. Katano, R. Ito, M. Ito, M. Minegishi, N. Minegishi, S. Tsuchiya, and K. Sugamura. 2009. The analysis of the functions of human B and T cells in humanized NOD/shi-scid/{gamma}cnull (NOG) mice (hu-HSC NOG mice). *Int Immunol*.


*from* Ito, M. *et al.* NOD/SCID/gamma(c)(null) mouse: an excellent recipient mouse model for engraftment of human cells. *Blood* 100, 3175-3182. (2002).

# *Development of lymphoid lineage cells in NOG mice transferred with human CD34+ cells*

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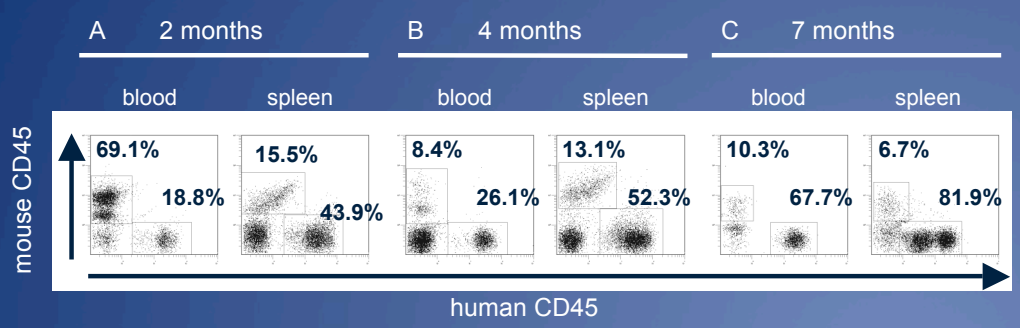
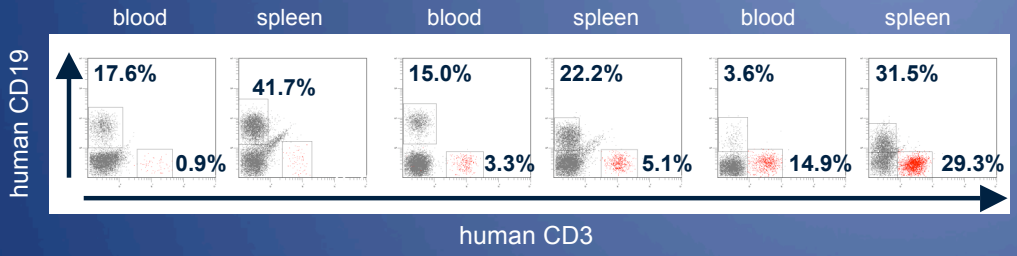
## Leukocytes

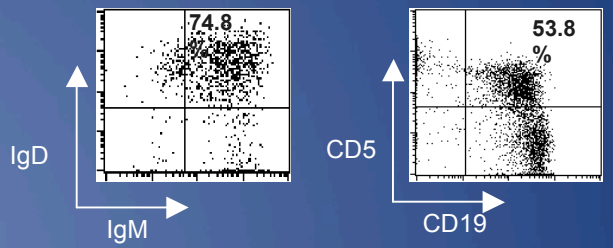



## T and B cells

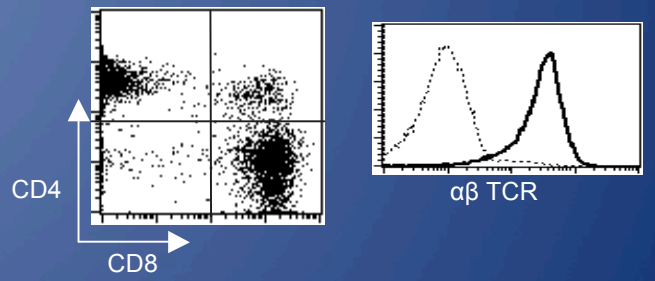



In the development of human cells from HSCs in NOG mice, B cells develop 4-8 weeks after transfer. T cells can be detected in 8-12 weeks after transfer and later become dominant in the periphery including peripheral blood and spleen.

## B cell characters




## T cell characters




The above figure shows human cells in mouse peripheral blood 20 weeks after intravenous human HSC cell transfer. Human T and B cells were developed in NOG mice. IgM+IgD+ B cells and CD4+ and CD8+ T cells were also differentiated in NOG mice.

*from* Dr. Habu S, Tokai Univ.

*from* Watanabe, S. *et al.* Hematopoietic stem cell-engrafted NOD/SCID/IL2Rgamma null mice develop human lymphoid systems and induce long-lasting HIV-1 infection with specific humoral immune responses. *Blood* 109, 212-218 (2007).

# *Development of myeloid lineage cells in NOG mice transferred with human CD34+ cells*

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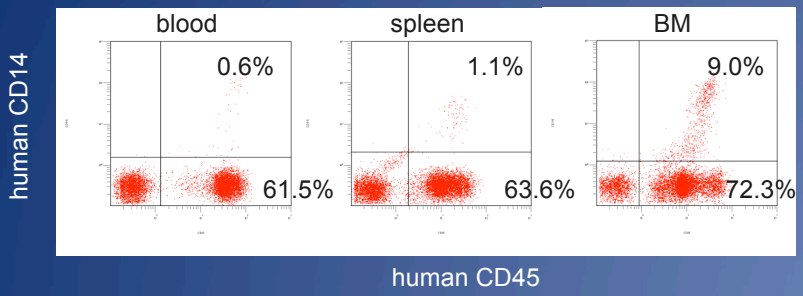
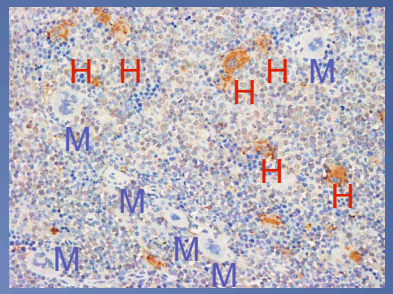
## Monocyte




## Megakaryocyte

In bone marrow

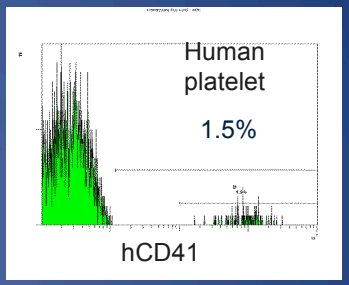



hCD42b staining

H; human megakaryocytes
M; mouse megakaryocytes

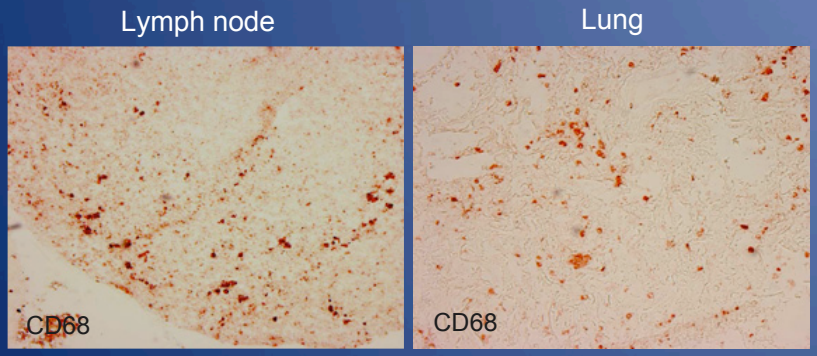
## Platelet

In peripheral blood

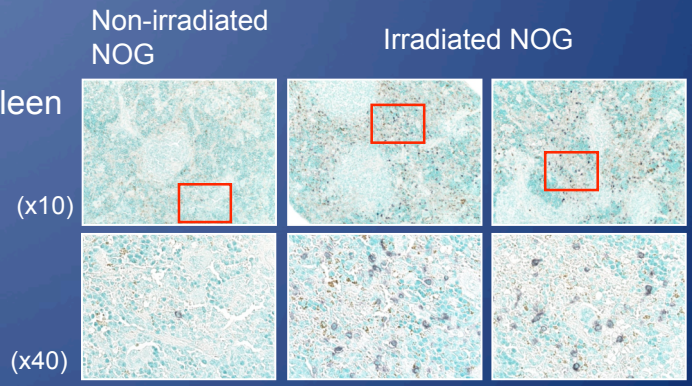



*from* Dr. Miyakawa Y, Keio Univ.

## Macrophage




## Mast cell




hTryptase staining

*from* Watanabe, S. *et al.* Hematopoietic stem cell-engrafted NOD/SCID/IL2Rgamma null mice develop human lymphoid systems and induce long-lasting HIV-1 infection with specific humoral immune responses. *Blood* 109, 212-218 (2007).

# *Development of lymphoid follicle in NOG mice reconstituted human CD34+ cells*

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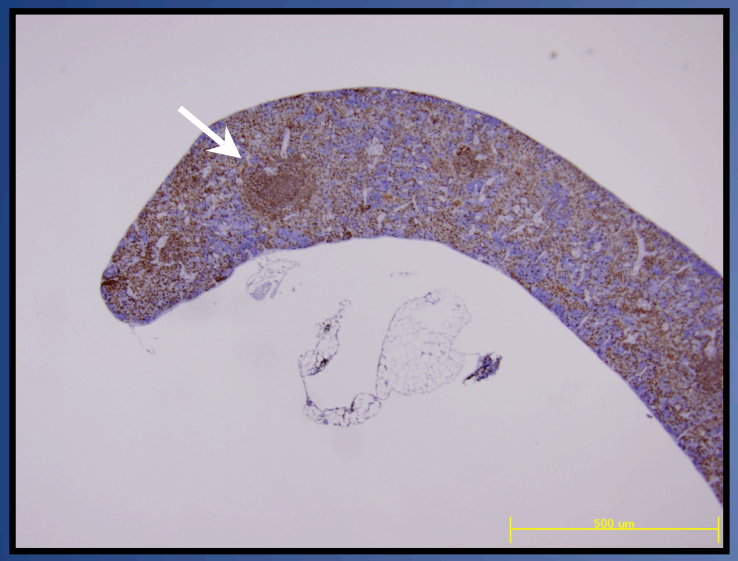
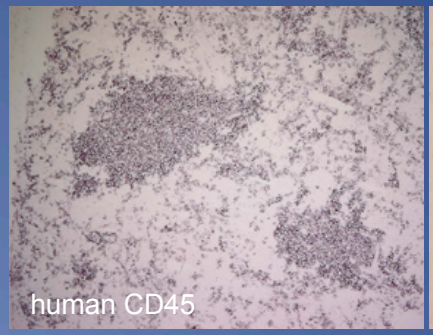
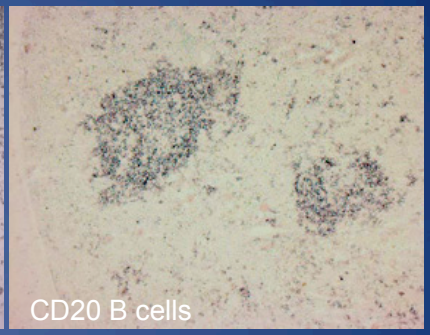
Spleen

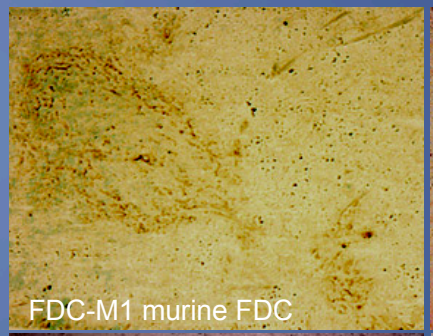


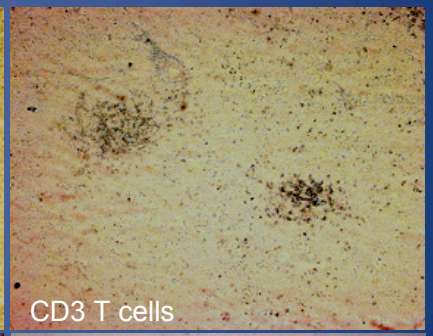
HLA staining

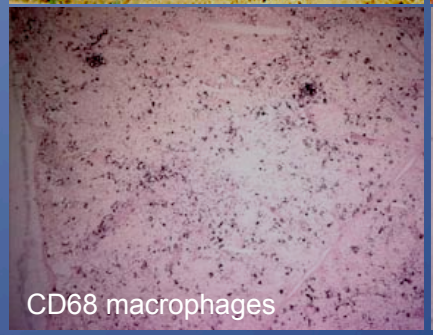
Human lymphoid follicle like structures (White arrow) were observed in spleen of NOG mice 18 wks after human HSC transfer.

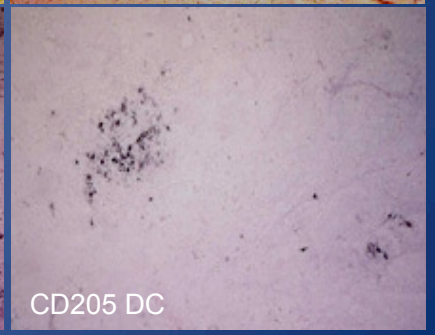



Human B, T cells and other monocytes/macrophages and DCs, but not FDCs were associated with the generation of lymphoid follicle-like structures observed in spleen.

*from* Watanabe, S. *et al.* Hematopoietic stem cell-engrafted NOD/SCID/IL2Rgamma null mice develop human lymphoid systems and induce long-lasting HIV-1 infection with specific humoral immune responses. *Blood* 109, 212-218 (2007).

# *Analysis of T cell receptor Vβ repertoire*

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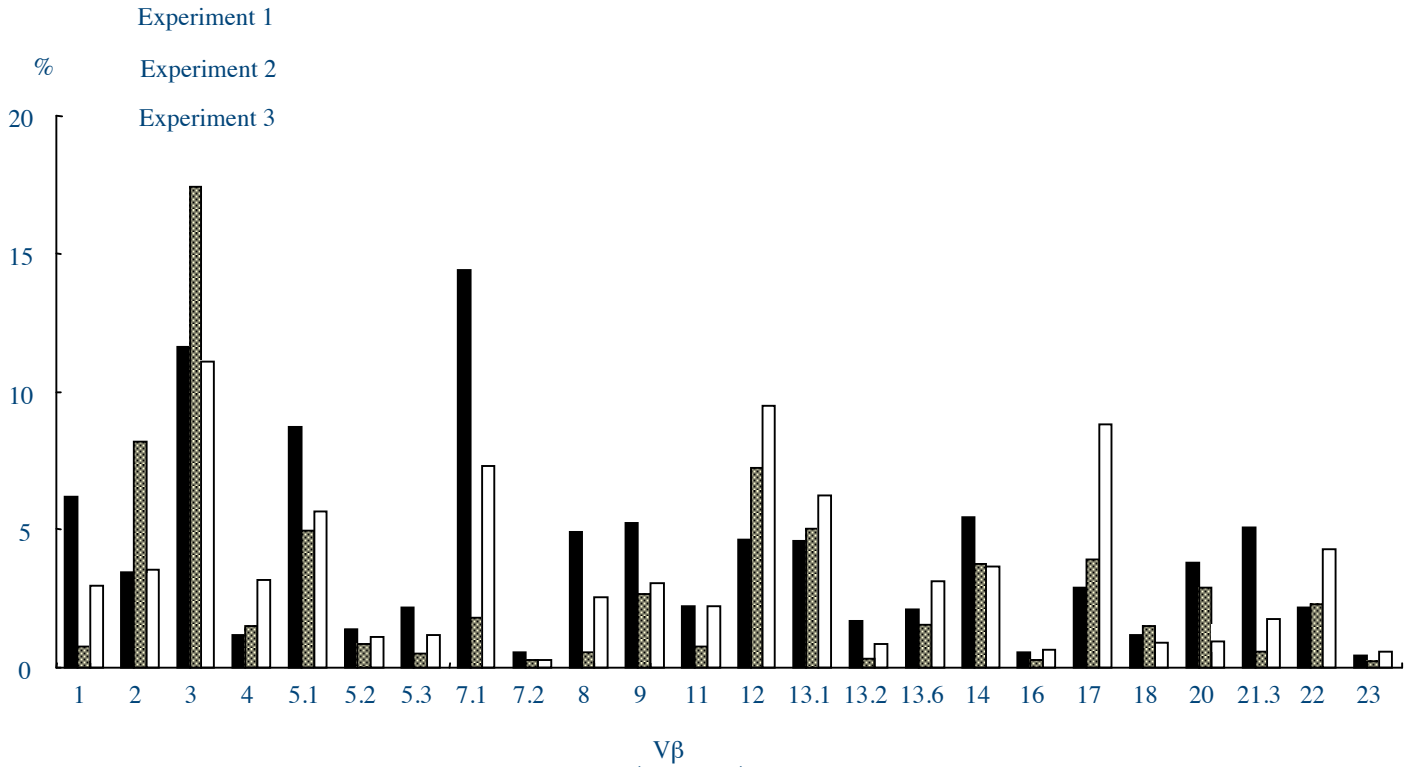
TCR Vß repertoire analysis of human T cells in spleen.




Four to six months after transplantation of $2\times10^4$ to $5\times10^4$ CB $CD34^+$ cells, spleen cells were taken and the TCR Vß repertoire was analyzed by flow cytometry using a panel of 24 different antibodies. The results of three independent experiments are shown.

*from* Hiramatsu, H., R. Nishikomori, T. Heike, M. Ito, K. Kobayashi, K. Katamura, and T. Nakahata. 2003. Complete reconstitution of human lymphocytes from cord blood CD34+ cells using the NOD/SCID/gammacnull mice model. *Blood* 102:873-880.

# *Activation of human T cells developed in NOG mice*

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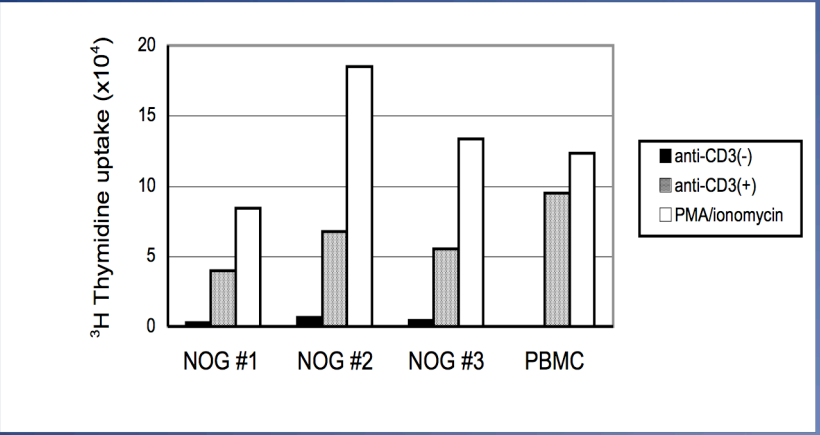
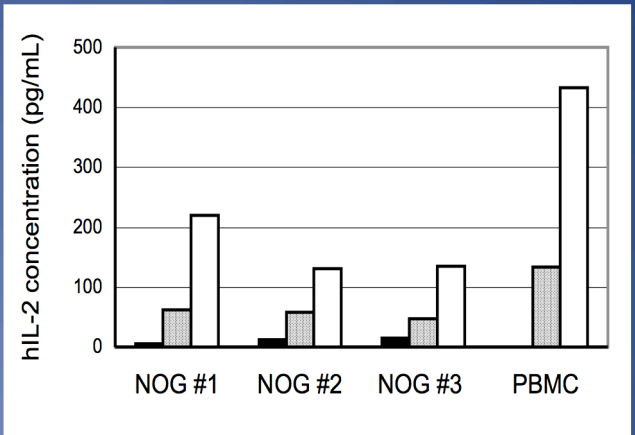
## Proliferation and cytokine responses

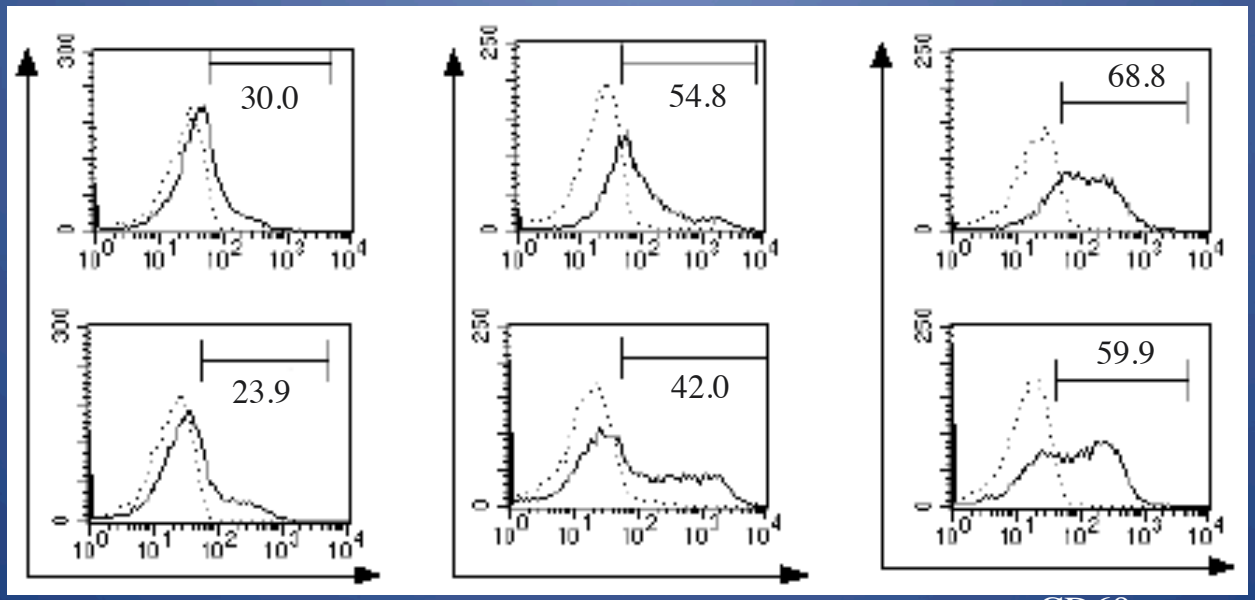



## Activation markers

hCD4 T cell from NOG spleen

hCD4 T cell from hPBMC




*from* Dr. Habu S, Tokai Univ.

# *Antibody production in NOG mice transferred human HSCs*

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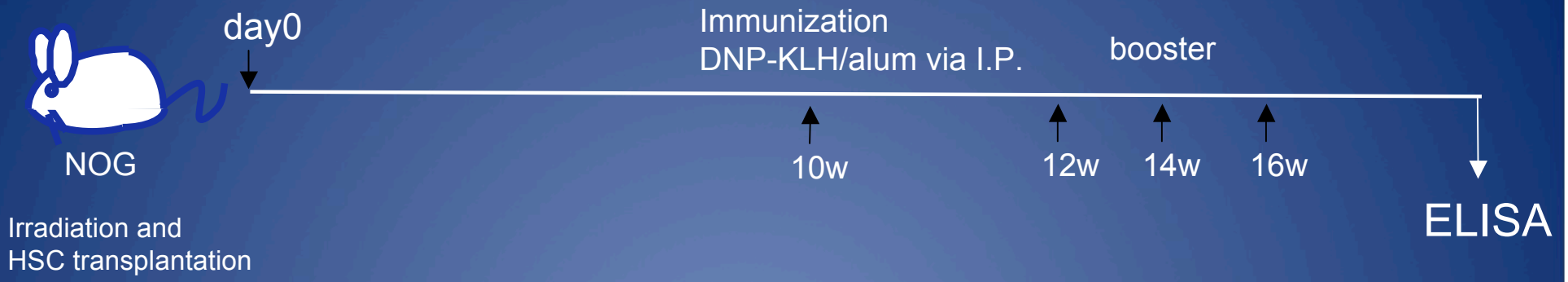
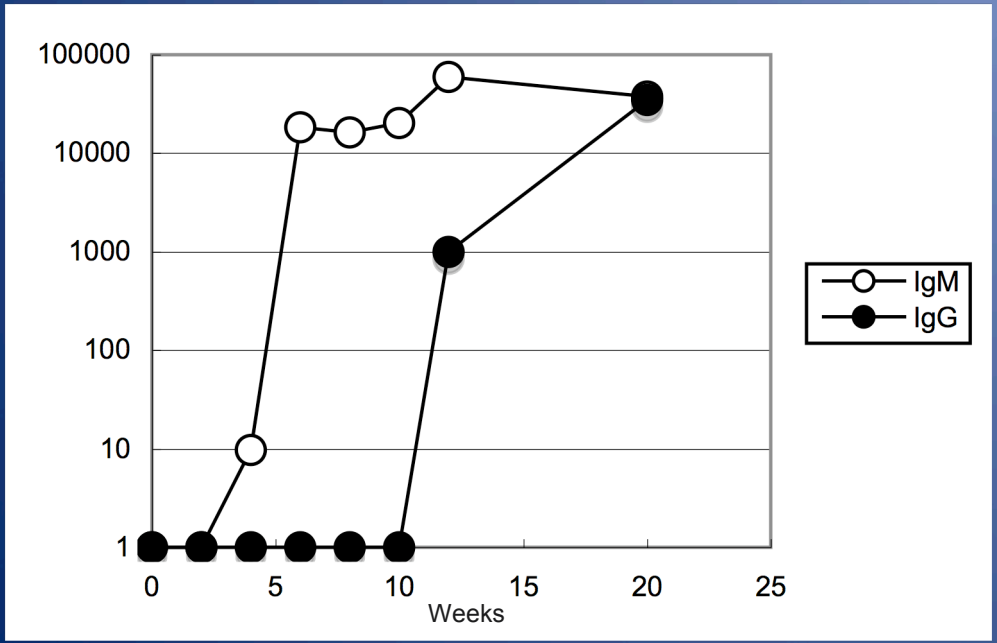
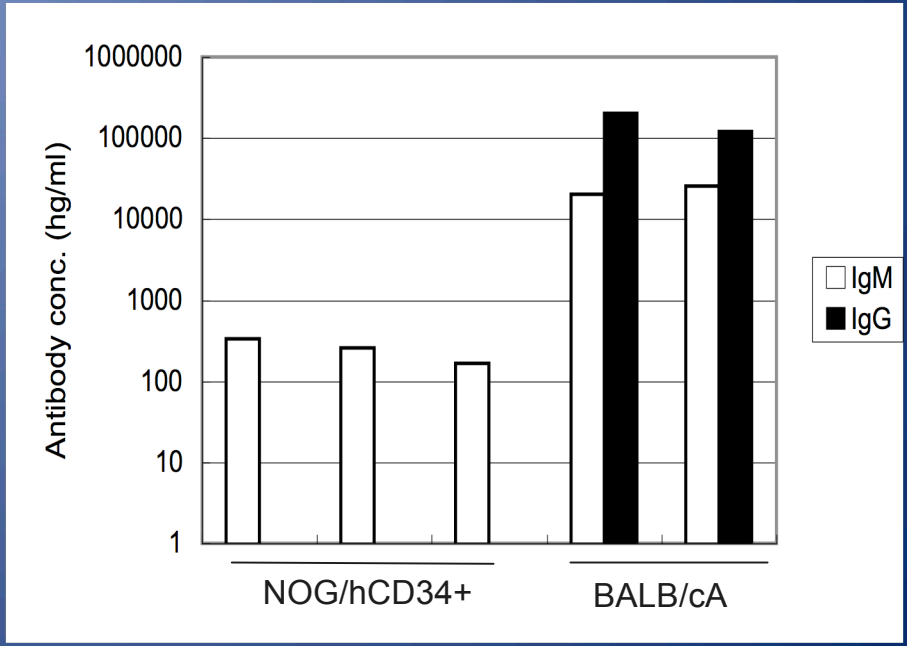



Non-specific antibodies




Anti-DNP-KLH specific antibodies




*from* Dr. Habu S, Tokai Univ.

# *Summary of human hematopoietic cells differentiated from CB CD34⁺ cells in NOG mice*

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