Retroviral transduction in cultured murine basophils

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Method Article

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Abstract

Introduction

Basophils are one of effector cells in type 2 immune responses that critically are associated with allergic inflammation and infections with helminth parasites. Recent findings that basophils produce a large amount of Th2 cytokines \(IL-4 and IL-6) has provided new insights into the possible role of basophils in the initiation phase, in addition to the effector phase, of type 2 immune responses. However, basophils occupy only 1% or less in blood, bone marrow and spleen, making it difficult to analyze their function. Here we establish a method for expressing exogenous proteins and their mutants in cultured basophils through retroviral infection. In combination with cultured basophils from gene-manipulated mice, this method, overcoming the paucity of basophils, allows us to examine the requirement of various signal transducers in interleukin 3-induced production of Th2 cytokines.

Reagents

– RMPI 1640 – Fetal bovine serum \(FBS) – Recombinant mouse interleukin-3 – Biotin-conjugated anti-c-kit antibody \(e-Bioscience) – Biotin-conjugated anti-rat CD2 \(rCD2) antibody \(Cedarlane) – PE-Cy7-streptavidin \(BD Biosciences) – RetroNectin \(TakaraBio) – FuGene-6 reagent \(Roche Diagnostics) – pMX-IRES-rCD2 \(The GFP portion in the original pMX-IRES-GFP vector was replaced with the extracellular-transmembrane domain of rCD2; see ref. 1)

Equipment

– Flowcytometer \(FC500, Beckman-Coulter) – BD IMag^{TM} Cell Separation System \(BD Bioscience) – AutoMACS \(Myltenyi Biotech)

Procedure

Preparation of retrovirus-containing supernatant 1. Transfect retroviral constructs \(based on the bicistronic vector, pMX-IRES-rCD2, see ref. 1) into the packaging cell line Phoenix using FuGene-6 reagent as instructed by supplier. 2. Collect retrovirus-containing supernatants 48 hours after transfection, and concentrate 10-fold by centrifugation \(8000 g, 12 hours, 4°C). 3. Filtrate virus supernatants through 0.22μm pore PVDF membrane. The filtrated supernatants can be stored for up to one week at 4°C. **Infection of BM-derived basophils with retroviruses** 4. Isolate bone marrow \(BM) cells from femurs and tibias of 8-12 week old mice. 5. Culture whole BM cells at 2 to 2.5 × 10^6 cells/ml in 10 ml of penicillin and streptomycin, 2mM L-glutamine, 50 μM β-mercaptoethanol, 10% fetal bovine serum-containing RPMI 1640 medium supplemented with recombinant mouse IL-3 \(5 ng/ml) for 9 days with medium changed every 3 days. 6. Enrich BM-derived basophils by depleting mast \(c-kit$^+$) cells using IMag system or autoMACS as instructed by the supplier. 7. Treat 12-well plates with RetroNectin solution \(50 μg/ml in PBS) for 2 hours at room temperature, followed by 2% bovine serum albumin in PBS for 30 minutes. 8.

Incubate the treated plates with the virus supernatants for 4 hours at 30°C. Add enriched BM-derived basophils \(0.5 to 1 × 10^6 cells/ml) and culture for 2 days for infection. 9. Recover infected cells and wash with RPMI 1640 containing 2% FBS. 10. For stimulating with IL-3, infected BM-derived basophils are ‘starved’ by culturing in culture media without IL-3 for 12 to 18 hours before enrich rat $CD2^+$ cells with IMag system and/or AutoMACS.

Anticipated Results

See Figure 1.

References

1. Yamasaki, S. _et al_. Mechanistic basis of pre-T cell receptor-mediated autonomous signaling critical for thymocyte development. _Nat. Immunol._ **7**, 67-85 \(2006).

Figures

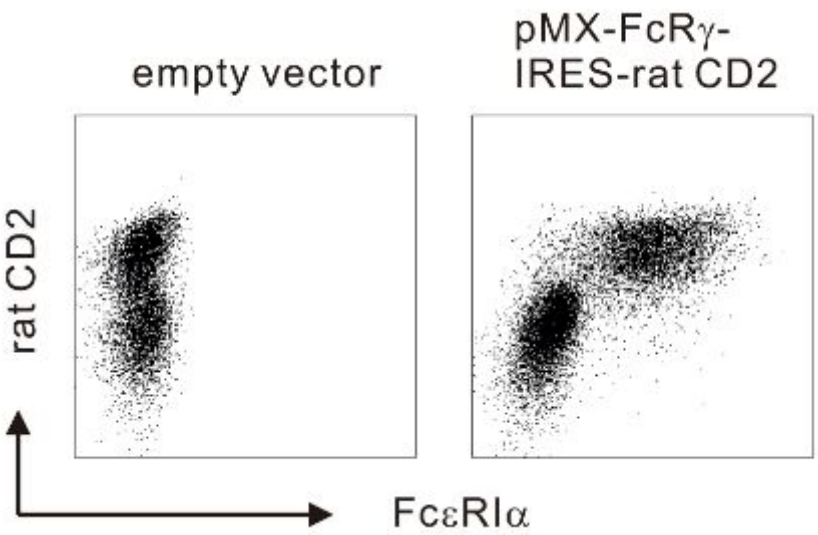

Figure 1

Retroviral reconstitution of FcRγ allows surface expression of FcεRI on BM-derived basophils. BM-derived basophils from FcRγ-deficient mice were infected with either control or FcRγ-expressing retrovirus. Infected cell, deleted of c-kit^+^ cells, were stained with anti-FcεRIα (MAR-1, e-Bioscience) and anti-rat CD2 (OX34, Cedarlane) and analyzed on the FC500 flowcytometer.