Method for selecting and enriching cells expressing low affinity ligands for cell surface receptors

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The affinity of protein-protein interaction in biological systems ranges from extremely strong, such as that between avidin and biotin (K_a = approximately 10^{15} M⁻¹) to the very weak, such as E-selectin and E-selectin ligand-1 (K_a = approximately 7.4×10^4 M⁻¹S⁻¹) (1). It is known that the interactions mediated by the surface receptors of immune cells are often of low-affinity and transient in nature (2). Despite weak affinities, cell surface interactions among immune cells have been shown to play an important role in many functions such as surveillance, immunosynapse formation, and activation (2). It is therefore desirable to devise a way to select and enrich cells expressing low-affinity ligands for specific immune cell surface receptors. Here we use the well-studied interaction between CD97 and CD55 as a model system to describe a simple method for this purpose.

Both CD97 and CD55 are cell surface proteins expressed predominantly on leukocytes (3–5). The binding of CD97 to CD55 has been demonstrated to be both of low affinity (86 μ M) and transient (off-rate; 0.6/s) (6). We and others have previously shown that the interaction is mediated exclusively by the epidermal growth factor (EGF)-like domains of CD97 and the short consensus repeats (SCR) of CD55 in a Ca²⁺-dependent fashion (6,7). Furthermore, a CD97-like molecule, EMR2, has been shown not to bind CD55 even though it contains highly homologous EGF-like domains (97.5% identity), therefore providing an ideal negative control (8).

Our method is based on (i) the efficient isolation and coupling of Fc-fusion proteins to protein AG-conjugated paramagnetic beads (Protein A/G Dynabeads®; Dynal A.S., Oslo, Norway) (Figure 1A) and (ii) the simple capture and separation of ligand-positive cells from the Fc-fusion protein-coated paramagnetic beads (Figure 1B). The coupling of the Fc-fusion proteins to the protein AG-conjugated paramagnetic beads

generates a multivalent protein probe, thereby increasing binding efficiency (Figure 1, A and B). As the major CD55-binding CD97 isoform contains three EGF-domains, we fused the three EGF-domains immediately upstream of the mouse Fc region (Figure 1A). CD97(EGF-1,2,5)-mFc and EMR2(EGF-1,2,5)-mFc fusion proteins were produced as described previously in transiently transfected HEK293T cells (Figure 1C).

Protein AG-conjugated paramagnetic beads were washed three times in binding buffer [0.5% bovine serum albumin (BSA) in Hank's Balanced Salt solution (HBSS)], reconstituted in the binding buffer to the original concentration (10 mg/mL), and incubated at 4°C for 30 min with or without 2 μ g mFc-fusion protein/10 μ L beads/reaction. Following the removal of unbound proteins by magnetic separation, the fusion protein-bead complexes were then resuspended in 20 μ L binding buffer/reaction. K562 cells (CD55⁺) were washed, adjusted to a cell density of 3×10^6 cells/mL, and incubated at 4°C with the protein-bead

Figure 1. The selection of low-affinity ligand-bearing cells. (A) Representation of the mFc-fusion proteins and the protein-bead complex. The epidermal growth factor (EGF)-like modules and the mFc region are represented by triangles and half-circles, respectively. The big gray circle represents protein AG-conjugated paramagnetic bead. The mFc-fusion protein is shown as a dimer. (B) Diagrams depicting the selection and enrichment of ligand-bearing cells using the mFc-fusion protein-bead complex. (C) Western blot analysis of CD97(1,2,5)-mFc and EMR2(1,2,5)-mFc fusion proteins. An Fc-specific anti-mouse immunoglobulin G horseradish peroxidase (IgG-HRP) was used to detect the mFc-fusion proteins: lane 1, mock transfected conditioned medium; lane 2, CD97(1,2,5)-mFc; and lane 3, EMR2(1,2,5)-mFc. The mFc fusion proteins were then purified and bound to the protein AG-conjugated paramagnetic bead to form the protein-bead probes.

Figure 2. The binding and selection of CD55-bearing cells using CD97-beads. CD55⁺-K562 cells were shown to be bound by CD97-beads (A, arrow) but not EMR2-beads (B). CD55⁺-cells can be selected and enriched from CD97-beads (E and F) but not EMR2-beads (C and D), either from a homogenous cell population such as K562 cells (C and E) or from a mixed cell population such as blood (D and F).

complexes (20 μ L/mL cell solution/reaction) for 1 h on an end-over-end rotator. When examined under the microscope, it was clear that K562 cells bound to CD97-bead but not EMR2-bead complexes (Figure 2, A and B). The cell-bead mixture was then washed three times gently with the binding buffer, and the unbound cells were removed by magnetic separation. Bead-bound cells were separated and isolated from the beads by incubation in binding buffer containing 5 mM EDTA for 10 min at room temperature followed by magnetic separation (Figure 2, C and E). The total number of recovered cells was determined by hemocytometer counting. We found that while CD97-coated beads bind and capture approximately 15% of input cells (approximately $4.5 \times 10^5/3 \times 10^6$), control beads (EMR2-coated beads as well as beads alone) do not capture any significant number of cells at all (data not shown). Further studies have shown that the number of captured cells depends on the number of the input cells in a dose-dependent manner with the maximum capacity reaching approximately 1×10^7 cells/10 μ L beads (data not shown). In addition, we have found that the protein-bead complexes can be reused several times with no apparent loss of binding efficiency (data not shown).

beads. We further confirmed that the binding and isolation of red blood cells were indeed mediated by CD97-CD55 interaction, as the addition of anti-CD55 antibody almost completely abolished the ability of CD97-coated beads to capture red blood cells (data not shown). Attempts to directly capture soluble CD55 ligands from K562 cell lysate using the CD97-coated beads were unsuccessful (data not shown). This is most likely due to the extremely weak and transient interaction between CD97 and CD55 and further supports the importance of multivalency in detecting low affinity receptor-ligand interaction.

In summary, we have described a method that allows the selection and enrichment of cells that express cellular ligands involved in the low-affinity interaction with specific cell surface receptors. This method provides an efficient way to produce multivalent protein probes with high binding efficiency, allowing for a simple procedure to select and enrich ligand-bearing cells. This method is useful not only in characterizing receptor-ligand interaction, but also in providing an enriched source of ligand-bearing cells for downstream applications, such as the construction of expression libraries to identify unknown ligands.

To test whether this method could also work in a mixed cell population, freshly obtained human whole blood was mixed with protein-coated beads. CD55-positive red blood cells were captured and isolated. Figure 2, D and F, show that a significantly pure population of red blood cells was obtained from CD97-coated beads, while no cells were purified by EMR2-coated

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COMPETING INTERESTS STATEMENT

The authors declare no competing interests.

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