EFFECTS OF MEMBRANE ANCHOR AND GLYCOSYLATION ON FCγ RECEPTOR III BINDING TO HUMAN IGG1

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INTRODUCTION

Low affinity Fcγ receptor III (CD16) is expressed on almost all hematopoietic cells. Binding to Fc of multimeric IgG in immune complexes cross-links CD16, which triggers a wide variety of immune responses depending on the cells on which it expresses. CD16 has two membrane isoforms, CD16a with a transmembrane (TM) anchor ($CD16a^{TM}$) and two alleles of CD16b with a glycosylphosphatidy-linositol (GPI) anchor ($CD16b^{NA1}$ and $CD16b^{NA2}$). Although differing by only a few amino acids in the extracellular domain, CD16 isoforms have varying degrees of glycosylation and bind to IgG Fc with distinct kinetic rates and affinity. We used the micropipette adhesion frequency assay [1] to measure the kinetic rates and affinities of human IgG1 binding of a soluble glycosylated CD16a-Ig chimera [2] and a soluble aglycosylated $CD16b^{NA2}$ [3]. These data are compared with previous results of cell surface CD16a [4] and $CD16b^{NA2}$ [5,6] measured by the micropipette and of soluble glycosylated CD16a-Ig [7] and soluble aglycosylated $CD16b^{NA2}$ measured by the surface plasmon resonance (SPR) [3,8] techniques.

MATERIALS AND METHODS

Aglycosylated $sCD16b^{NA2}$ was a generous gift of Dr. Catherine Sautes-Fridman [3]. Glycosylated sCD16a was a dimeric Ig chimeric molecule produced in house [2]. sCD16 was captured on microspheres (7.5 μm in diameter) using a nonblocking anti-CD16 monoclonal antibody (mAb) 214.1 (a generous gift of Dr. Howard Fleit), which was covalently coupled to the microspheres using carbodiimide chemistry. Human IgG1 (hIgG1) was coated on the surface of freshly isolated human red blood cell (RBC) using chromium chloride coupling [1,4-6]. CD16 and hIgG1 site densities on the respective microsphere and RBC surfaces were measured via flow cytometry using calibration beads [1,4-6].

Observing through a chamber mounted on the stage of an inverted microscope, a sCD16-coated microsphere and a hIgG1-coated RBC were captured by two apposing micropipettes (2-3 μm in inner diameter) aligned with a small axial gap between them. They were brought into contact in a controlled area for a prescribed duration and then separated. The occurrence of adhesion at the time the cell was retracted away from the microsphere was observed if the RBC apex remained adherent to the microscphere, leading to membrane elongation. For each contact time, five microsphere-RBC pairs were tested for a hundred contact-retraction cycles each to estimate the mean ± SEM of the adhesion frequency [1,4-6].

RESULTS

Adhesion frequencies P_a of sCD16 to hIgG1 measured in a range of contact time t were combined with the densities to produce a plot shown in Fig. 1. These data were then fit to the following equation [4]:

$$y = (m_r m_l)^{-1} \ln(1 - P_a)^{-1} = A_c K_a^0 [1 - \exp\{-k_r^0 t)] \qquad (1)$$

where m_r and m_l are the respective densities of receptors and ligands, A_c is the contact area, K_a^0 is the affinity, and k_r^0 is the reverse-rate. Plotting the data according to Eq. 1 allows the effective affinity and the reverse-rate to be visualized, respectively, from the steady-state level, $A_c K_a^0 = y(\infty)$, and the half-time (i.e., time to achieve half of the steady-state level), $k_r^0 = \ln 2 / t_{1/2}$, of the binding curve. As can be seen, sCD16a has a 2-3-folds higher affinity for hIgG1 than $sCD16b^{NA2}$, but $sCD16b^{NA2}$ may have a slightly faster reverse-rate. The best-fit parameters were listed in Table 1 along with previous results.

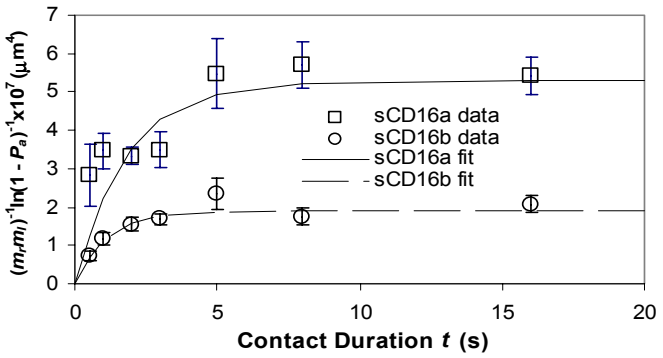

Figure 1. hIgG1 binding curves of sCD16a and $sCD16b^{NA2}$.

Table 1. Kinetic and equilibrium association constants of various CD16 molecules interacting with hIgG1 or total hIgG (*)

Receptor	Assay	k_r (s^{-1})	AcK_f ($10^{-6}\mu m^4 s^{-1}$)	AcK_a ($10^{-6}\mu m^4$)	Reference
Glycosylated sCD16a	MP	0.69	0.34	0.49	This work
Glycosylated CHO CD16a^{TM}	MP	0.34± 0.05*	0.25 ± 0.11*	0.74± 0.3*	[4]
Glycosylated CHO CD16a^{GPI}	MP	0.42± 0.02*	0.77 ± 0.29*	1.8± 0.7*	[4]
Aglycosylated sCD16b^{NA2}	MP	0.98	0.18	0.19	This work
Glycosylated CHO CD16b^{NA2}	MP	0.70 ± 0.11	0.28 ± 0.046	0.40 ± 0.02	[5]
Glycosylated K562 CD16b^{NA2}	MP	0.50 ± 0.06*	0.21 ± 0.027*	0.41 ± 0.02*	[6]
		k_r (s^{-1})	k_f ($\mu M^{-1}s^{-1}$)	K_a (μM^{-1})	
Glycosylated sCD16a	SPR	0.0057 ± 0.00036	0.00818 ± 0.00028	1.41 ± 0.08	[7]
Glycosylated sCD16b^{NA2}	SPR	0.00098± 0.0003	0.00113 ± 0.0003	1.3 ± 0.6	[3]
Aglycosylated sCD16b^{NA2}	SPR	0.00193± 0.0002	0.0021± 0.0003	1.1± 0.2	[3]
Aglycosylated sCD16b^{NA2}	SPR	1.4 ± 0.013	0.54 ± 0.016	0.40 ± 0.013	[8]

DISCUSSION

The primary goal of this study is to determine how the membrane anchor and glycosylation of CD16 affect its ligand binding. Previous studies have shown that CHO cell CD16a^{GPI} had a 3-fold higher affinity for hIgG than CHO cell CD16a^{TM} and a 6-fold higher affinity for hIgG than CHO cell CD16b^{NA2}, which also is GPI-anchored [4-6]. The sCD16a/hIgG1 effective affinity measured from the present work is similar to that of the previous CD16a^{TM}/hIgG data. Since the extracellular domains of CD16a^{GPI} and CD16a^{TM} are identical, which are also identical to the CD16a portion of the sCD16a-Ig chimera that is fused with a hIgG1 Fc [2], these data isolate the GPI anchor as the likely cause of affinity difference rather than the TM anchor, the lack thereof, or the fusion of it to IgG1 Fc.

If the GPI anchor was indeed primarily responsible for the difference between the hIgG1 binding affinities of CHO cell CD16b^{NA2} and sCD16b^{NA2}, then the glycosylation difference between the two molecules would have little effect. This is consistent with the previous report using the SPR technique to analyze hIgG1 binding of a glycosylated and an aglycosylated forms of sCD16^{NA2} [3].

The differences in the kinetic rates and affinities for hIgG (and hIgG1) of various CD16 molecules measured by the micropipette are modest. By comparison, those measured by SPR exhibit substantial discrepancies (Table 1). This is most evident in the results reported by references 3 and 8, which used the same sCD16b^{NA2} that showed identical crystal structures [9,10].

The micropipette adhesion frequency assay makes in situ measurements of receptors and ligands bound to apposing cell surfaces, resulting in the so-called two-dimensional (2D) kinetic parameters. By comparison, in the SPR experiment one molecule is immobilized on the sensor surface but the other molecule is flowing in solution, resulting in 3D kinetics. As such, the units for affinity are different - the 2D effective affinity $A_cK_a^0$ is reported in the unit of μm^4, and the 3D affinity K_a is reported in the unit of μM^{-1} – hence they cannot be directly compared. On the other hand, the reverse-rates are of the same units in both the 2D and 3D assays, and there have been reports suggesting agreements between 2D and 3D reverse-rates. While the 2D k_r^0 of sCD16^{NA2} dissociating from hIgG1 measured by the micropipette (present work) is similar to one of the 3D k_r measured by SPR [8], those of sCD16a measured from the two assays differ dramatically, despite the fact that identical sCD16a were captured by 214.1 in both assays. Clearly, additional work is required to sort out these questions.

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