

Antibody Fc Engineering for Optimal Engagement of Innate Immune Cells

John Richards, Greg Lazar, Sher Karki, Sreenivas Laxmanan, Aaron Chamberlain, & John Desjarlais



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Xencor, Inc. 111 W. Lemon Ave., Monrovia, CA 91016

Abstract

Antibody Fc gamma receptors (FcγRs) are present on a wide variety of effector cell populations, including NK cells, dendritic cells (DC), neutrophils, and macrophages. We have previously engineered the Fc domains of antibodies to enhance their affinity for FcγRIIIa, leading to dramatic enhancements in ADCC. While NK cell-mediated lysis occurs predominantly through a single activating receptor FcγRIIIa, activation of antigen presenting cells such as DC and macrophages can also be influenced by the activating receptor FcγRIIIa and inhibitory receptor FcγRIIb. In an effort to optimally tune antibodies for effector function, we have engineered a panel of Fc variants with a variety of unique FcγR affinities and specificities, including selective engagement of FcγRIIIa and FcγRIIa over FcγRIIb. Results indicate that whereas NK cell-mediated killing is correlated strongly with FcγRIIIa affinity, phagocytosis by macrophages is dependent on binding to both FcγRIIIa and FcγRIIIa. Surprisingly, our data show that absolute FcγRIIb affinity does not negatively impact phagocytosis. Overall these variants have the potential to improve anti-cancer therapy by increasing not only innate effector functions, but also by enhancing adaptive anti-tumor responses, a novel feature of engineered therapeutic antibodies.

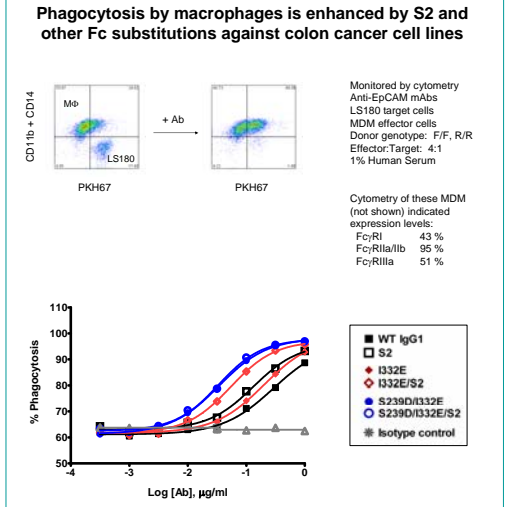
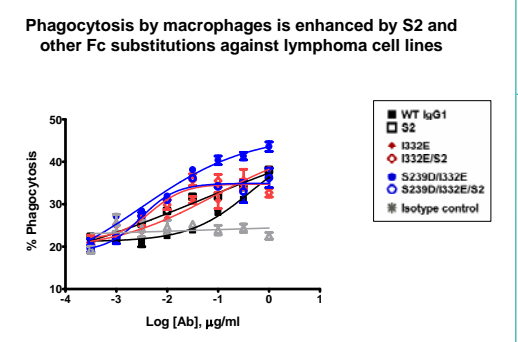
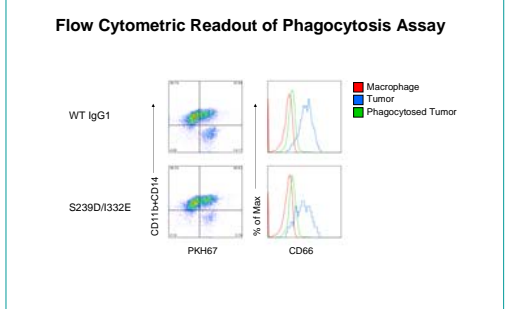
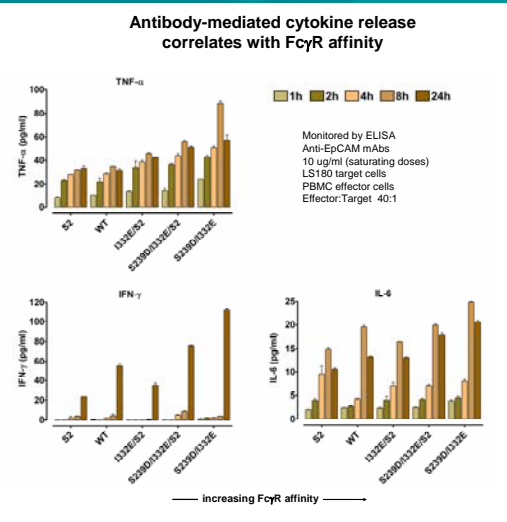
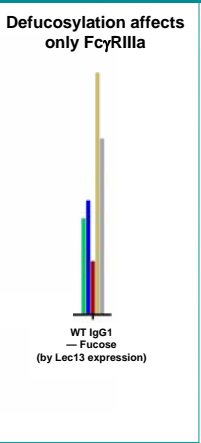
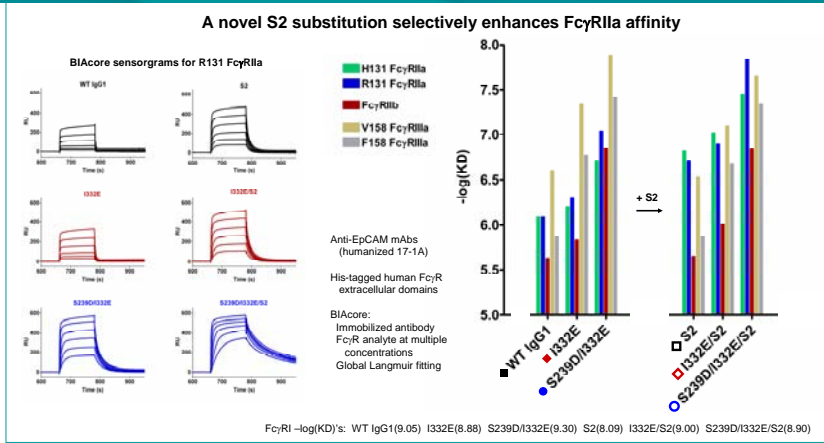
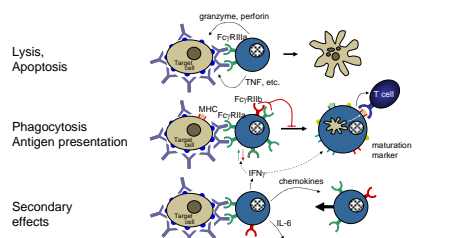
Effector cells, effector functions, and activating vs. inhibitory FcγRs

Cell type	Anti-tumor relevance		FcγR-dependent function(s)	FcγR expression
	colon, breast, lung, ovarian	lymphomas		
NK	low?	high?	ADCC	Illa
Neutrophil	?	high?	Phagocytosis ADCC	[I, IIa] vs [IIb, IIIb]
Macrophage	high?	?	Phagocytosis ADCC	[I, IIa, IIIa] vs [IIb] [I, IIa, IIIa] vs [IIb]
DC	high?	?	Phagocytosis Antigen presentation	IIa vs IIb

Literature supporting relevance of FcγRIIIa and monocyte-derived effector cells

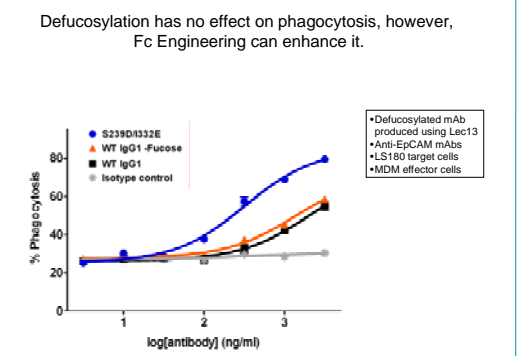
- Higher FcγRIIIa affinity leads to longer progression free survival in anti-GD2 neuroblastoma therapy (Cheung et al., JCO 2006)
- FcγRIIIa-mediated phagocytosis by human DCs leads to enhanced DC maturation and CTL priming (Dhodapkar et al., J Exp Med 2002; Groh et al., PNAS 2005; Boruchov et al., J Clin Invest 2005)
- Antibody efficacy in murine models
 - Dependent on monocyte population (Hamaguchi et al., J Exp Med 2006)
 - Dependent on FcγRIV, expressed on monocytes/macrophages (Nimmerjahn & Ravetch, Science 2005)
 - Dependent on T cells (Dyall et al., Eur J Immunol 1999; Wolpoe et al., J Immunol 2003)

FcγR-mediated effector functions



Monitored by cytometry
Anti-CD19 mAbs
Raji target cells
MDM effector cells
Donor genotype: F/F, R/H
Effector:Target: 3:1
1% Human Serum

Cytometry of these MDM (not shown) indicated expression levels:
FcγRI 83 %
FcγRIIIa/IIb 98 %
FcγRIIIa 49 %



Summary score sheet for variant activities (Anti-EpCAM, LS180 target cells)

Cell type	FcγR	FcγR-dependent function(s)	S239D/I332E	I332E	WT	S2	I332E/S2	S239D/I332E/S2
PBMC	all	ADCC	+++	++	+	-	+	+++
NK	Illa	ADCC	+++	++	+	-	+	+++
Macrophage	I, IIa, IIIa IIb	Phagocytosis Cytokine release	+++	++	+	++	+++	+++
Neutrophil	I, IIa, IIIa IIb	Phagocytosis ADCC						in progress
moDC	IIa IIb	Maturation Cytokine release						in progress
Mutations			2	1	0	1	2	3

Summary

- Engineered Fc variants enhance a variety of FcγR-mediated effector functions
 - Enhanced ADCC by PBMC and/or NK cells
 - Enhanced phagocytosis by macrophages
 - Solid Tumors
 - Liquid Tumors
 - Enhanced cytokine release by PBMC
- A novel variant, S2, selectively increases FcγRIIIa affinity in multiple contexts
- Combination variants have broadly enhanced FcγR activities

Acknowledgements

We thank the following people for helpful discussions and technical assistance:
Bassil Dahiyat, Lori Kunkel, Phil Hammond, Eugene Zhukovsky, Wei Dang, Seung Chu, Hsing Chen, Anjali Simh
Madhav Dhodapkar, Lou Weiner, Raphael Clynes