Leukocyte immunoglobulin-like receptor B1 is critical for antibody-dependent dengue

Kuan Rong Chan^{a,b,1}, Eugenia Z. Ong^{a,1}, Hwee Cheng Tan^a, Summer Li-Xin Zhang^c, Qian Zhang^{a,d}, Kin Fai Tang^a, Nivashini Kaliaperumal^e, Angeline Pei Chiew Lim^c, Martin L. Hibberd^f, Soh Ha Chan^g, John E. Connolly^e, Manoj N. Krishnan^a, Shee Mei Lok^{a,d}, Brendon J. Hanson^{c,g}, Chao-Nan Lin^{a,h,2}, and Eng Eong Ooi^{a,c,g,i,3}

^aProgram in Emerging Infectious Diseases, Duke-NUS Graduate Medical School, Singapore 169857; ^bGraduate School for Integrative Sciences and Engineering, National University of Singapore, Singapore 117456; ^cBiological Defense Program, DSO National Laboratories, Singapore 117510; ^dCenter for Bioimaging Sciences, Department of Biological Sciences, National University of Singapore, Singapore 117557; ^eSingapore Immunology Network, Agency for Science, Technology and Research (A*STAR), Singapore 138648; ^fGenome Institute of Singapore, Agency for Science, Technology and Research (A*STAR), Singapore 138672; ^gDepartment of Microbiology, Yong Loo Lin School of Medicine, National University of Singapore, Singapore 117545; ^hDepartment of Veterinary Medicine, National Pingtung University of Science and Technology, Pingtung 912, Taiwan; and ⁱSaw Swee Hock School of Public Health, National University of Singapore, Singapore 117597

Edited by Rafi Ahmed, Emory University, Atlanta, GA, and approved December 20, 2013 (received for review September 16, 2013)

Viruses must evade the host innate defenses for replication and dengue is no exception. During secondary infection with a heterologous dengue virus (DENV) serotype, DENV is opsonized with sub- or nonneutralizing antibodies that enhance infection of monocytes, macrophages, and dendritic cells via the Fc-gamma receptor (FcγR), a process termed antibody-dependent enhancement of DENV infection. However, this enhancement of DENV infection is curious as cross-linking of activating FcγRs signals an early antiviral response by inducing the type-I IFN-stimulated genes (ISGs). Entry through activating FcγR would thus place DENV in an intracellular environment unfavorable for enhanced replication. Here we demonstrate that, to escape this antiviral response, antibody-opsonized DENV coligates leukocyte Ig-like receptor-B1 (LILRB1) to inhibit FcγR signaling for ISG expression. This immunoreceptor tyrosine-based inhibition motif-bearing receptor recruits Src homology phosphatase-1 to dephosphorylate spleen tyrosine kinase (Syk). As Syk is a key intermediate of FcγR signaling, LILRB1 coligation resulted in reduced ISG expression for enhanced DENV replication. Our findings suggest a unique mechanism for DENV to evade an early antiviral response for enhanced infection.

early innate immune response | innate immune signaling | immune evasion

Despite long-lived serotype-specific immunity upon initial infection, predicted global prevalence of dengue now surpasses World Health Organization estimates by more than threefold with 390 million cases annually (1). Furthermore, the risk of severe disease is augmented by cross-reactive or subneutralizing levels of antibody (2, 3), which opsonize dengue virus (DENV) to ligate Fc-gamma receptor (FcγR) for entry into monocytes, macrophages, and dendritic cells, a phenomenon known as antibody-dependent enhancement (ADE) of DENV infection (4, 5). The resultant greater viral burden leads to increased systemic inflammation that precipitates plasma leakage, a hallmark of dengue hemorrhagic fever (6). However, ligation of the activating FcγRs by immune complexes has been shown to induce type-I IFN stimulated genes (ISGs), independent of autocrine or paracrine IFN activity, unless the inhibitory FcγRIIB is coligated (7). We and others reported recently that coligation of FcγRIIB by DENV immune complexes requires high antibody concentration, and such coligation inhibited the entry of DENV immune complexes into monocytes (8, 9). At low antibody concentrations where ADE occurs, the inhibitory FcγRIIB is not coligated (9). Ligation of the activating FcγRs by DENV opsonized with subneutralizing levels of antibody would thus induce the expression of ISGs and hinder DENV replication (10). Here, we demonstrate that DENV employs a unique evasive mechanism by coligating LILRB1 to down-regulate the early antiviral responses triggered by activating FcγRs for ADE.

Results

ADE Differs in THP-1 Subclones. Our work was enabled by the isolation of subclones of THP-1 cells with different phenotypes to ADE. The low rate of FcγR-mediated phagocytosis in THP-1 cells (~5%) (9) had led us to reason that this cell line is genetically heterogeneous, either through the method in which it was derived (11) or through genetic instability resulting from aneuploidy (12). Screening of our newly isolated subclones with DiD (1, 1'-dioctadecyl-3, 3', 3' – tetramethylindodicarbocyanine, 4-chlorobenzenesulfonate salt) labeled DENV-2 alone or opsonized with subneutralizing concentrations of humanized 3H5 monoclonal antibody (h3H5) identified two clones (labeled as THP-1.2R and THP-1.2S) that showed increased uptake of DENV immune complexes compared with parental THP-1 (Fig. 1A). Monocyte surface marker analysis indicated no significant difference in the expression of FcγRs (FcγRI, FcγRII, FcγRIII) in these subclones (SI Appendix, Fig. S1A). Expression of FcγRIIA,

Significance

Dengue virus (DENV) infects almost 400 million people annually and some of these infections result in life threatening disease. An incomplete understanding of pathogenesis, particularly on how non- or subneutralizing levels of antibody augments DENV infection of cells expressing Fc-gamma receptors (FcγRs), has hampered vaccine development. Here, we show that, to overcome the activating FcγR-dependent expression of type-I interferon stimulated genes (ISGs), DENV binds and activates the inhibitory receptor, leukocyte immunoglobulin-like receptor-B1 (LILRB1). LILRB1 signals through its immunoreceptor tyrosine-based inhibition motif cytoplasmic tail to inhibit the expression of ISGs required for successful antibody-dependent DENV infection. Inhibition of DENV activation of LILRB1 could hence be a strategy for vaccine or therapeutic design.

Author contributions: K.R.C., E.Z.O., and E.E.O. designed research; K.R.C., E.Z.O., H.C.T., S.L.-X.Z., Q.Z., K.F.T., and N.K. performed research; A.P.C.L., M.L.H., S.H.C., J.E.C., M.N.K., S.M.L., B.J.H., and C.-N.L. contributed new reagents/analytic tools; K.R.C., E.Z.O., H.C.T., S.L.-X.Z., Q.Z., K.F.T., N.K., M.L.H., S.H.C., J.E.C., M.N.K., S.M.L., C.-N.L., and E.E.O. analyzed data; and K.R.C., E.Z.O., and E.E.O. wrote the paper.

The authors declare no conflict of interest.

This article is a PNAS Direct Submission.

Freely available online through the PNAS open access option.

See Commentary on page 2404.

¹K.R.C. and E.Z.O. contributed equally to this work.

²Present address: Department of Veterinary Medicine, National Pingtung University of Science and Technology, Pingtung 912, Taiwan.

³To whom correspondence should be addressed. E-mail: engeong.ooi@duke-nus.edu.sg.

This article contains supporting information online at www.pnas.org/lookup/suppl/doi:10.1073/pnas.1317454111/-DCSupplemental.

Fig. 1. ADE differs in THP-1 subclones. (A) Percentage of internalized DiD-labeled DENV-2 30 min postinfection under DENV-2 or ADE conditions in THP-1, THP-1.2R, and THP-1.2S. (B) Plaque titers of THP-1, THP-1.2R, or THP-1.2S when infected with DENV-2 opsonized with different h3H5 concentrations 72 h postinfection (hpi). Dotted lines indicate plaque titers following DENV-2-only infection, with no significant differences observed between the cell lines. (C) Time course of viral RNA copy numbers in THP-1.2R or THP-1.2S under ADE conditions. (D) Heat map showing fold change of ISG expression in THP-1.2R and THP-1.2S at 6 hpi under ADE conditions. (E) Fold change in transcript levels of interferons in THP-1.2R and THP-1.2S 6 hpi under ADE conditions. (F–I) Validation of microarray data in D by quantitative PCR. Data are expressed as mean $\pm$ SD from three independent experiments. ** $P < 0.01$, * $P < 0.05$.

FcγRIIB, and FcγRIIC were similar in these subclones (*SI Appendix, Fig. S1 B and C*). Both subclones were also heterozygous for 131 H/R FcγRIIA polymorphism (*SI Appendix, Fig. S1E*). Identical HLA haplotyping confirmed that both subclones were derived from THP-1 and not the result of a contamination with another cell line (*SI Appendix, Table S1*).

Despite no significant differences in uptake and production of plaque titers when infected with DENV-2 only, infection under ADE conditions resulted in significantly different DENV-2 titers in THP-1.2R and THP-1.2S (Fig. 1B). Similar observations were also made with enhancing titers of convalescent serum (*SI Appendix, Fig. S2A*) or other DENV serotypes (*SI Appendix, Fig. S2B*). Furthermore, early DENV RNA replication diverged in these two subclones, where a significant difference was observed as early as 6 h postinfection (Fig. 1C). Analysis of early gene expression indicated significant up-regulation of ISGs in THP-1.2R but not THP-1.2S (Fig. 1D and F–I). These included MX1, MX2, and viperin, which are potent inhibitors of DENV replication (10). The up-regulation of ISGs in THP-1.2R, however, was not due to h3H5 (*SI Appendix, Fig. S3*) and is independent of

IFN-α, -β, and -γ signaling as both subclones expressed similar IFN transcript levels (Fig. 1E). As expected, addition of antibodies that blocked IFNα receptor (IFNαR) signaling (*SI Appendix, Fig. S4A*) did not reduce this early ISG induction in THP-1.2R following infection (*SI Appendix, Fig. S4B*). The possibility that THP-1.2S had impaired IFNαR-mediated signaling was also excluded, as ISGs were significantly up-regulated in response to exogenous IFN (*SI Appendix, Fig. S4C*). These subclones thus serve as exquisite tools to decipher the signaling requirement to overcome the early antiviral responses for successful ADE.

Early ISG Expression During ADE Is Independent of RIG-I/MDA5 Signaling. Differences in viral entry through ADE and DENV-2-only conditions could have resulted in different intracellular antigenic load and hence resulted in differential ISG expression in the subclones. To identify the specific signaling pathway responsible for early ISG induction in THP-1.2R during ADE infection, we titrated the multiplicity of infection (MOI) for DENV-2 only that resulted in equivalent level of infection as ADE (MOI 10) to serve as an antigenically equivalent control

Fig. 3. Early ISG induction following ADE requires Syk phosphorylation. (A) Western blot and quantitative densitometry of pSyk levels using immunoprecipitation with Syk antibody. (B) ISG expression in DMSO- or piceatannol-treated (15.6 μ g/mL) THP-1.2R under DENV-2-only or ADE conditions 6 hpi. (C) Fold change in DENV RNA copy numbers in THP-1.2R and THP-1.2S pretreated with piceatannol relative to DMSO control. (D) Western blot, % LILRB1⁺ cells, and representative flow cytometry plots of LILRB1 in THP-1.2R and THP-1.2S. Cells were either stained with isotype (gray) or polyclonal anti-LILRB1 antibody (open histogram). (E) Western blot of pSHP-1, SHP-1 and GAPDH at different time points after infection under mock, DENV-2-only, and ADE conditions. (F) Quantitative densitometry of pSHP-1 levels under ADE conditions. Data are expressed as mean $\pm$ SD from three independent experiments. ** $P < 0.01$, * $P < 0.05$.

kinase inhibitor resulted in greater reduction of ISG expression under ADE conditions (Fig. 3B) and a correspondingly greater increase in DENV replication (Fig. 3C) compared with DENV-2 only. Increase in DENV replication was also greater in THP-1.2R than THP-1.2S. These findings suggest that early ISG expression in THP-1.2R is conditioned upon activating Fc γ R signaling through phosphorylated Syk (7).

Coligation of LILRB1 Inhibits ISG Induction. As activating Fc γ R signals through immunoreceptor tyrosine-based activation motif (ITAM), we postulated that DENV coligates an immunoreceptor tyrosine-based inhibition motif (ITIM)-bearing receptor to inhibit Syk activation (14) in THP-1.2S. Examination of the gene expression data identified two such possible receptors. LILRB1 (also known as CD85j or Ig-like transcript-2) and LILRB4 were up-regulated preinfection in THP-1.2S relative to THP-1.2R (SI Appendix, Fig. S5A). Flow cytometry analysis, however, showed that only LILRB1 (Fig. 3D and SI Appendix, Fig. S5B) displayed higher surface expression on THP-1.2S. Because one of the effects of ITIM phosphorylation is the recruitment and phosphorylation of SHP-1 (15, 16), we measured phosphorylated SHP-1 in the two subclones. Higher pSHP-1 levels were found in THP-1.2S than THP-1.2R under ADE conditions (Fig. 3E and F), suggesting that pSHP-1 dephosphorylated Syk in THP-1.2S.

If LILRB1 is necessary for ADE, then antibody-opsonized dengue should coligate LILRB1. Indeed, all four DENV serotypes bind to LILRB1, more strongly with whole virus than with E protein ectodomain (Fig. 4A and SI Appendix, Fig. S6A), suggesting that LILRB1 binds to a quaternary structure-dependent epitope. Furthermore, the addition of soluble extracellular domain of LILRB1 (SI Appendix, Fig. S6B) successfully competed with native LILRB1 on THP-1.2S to reduce ADE but not DENV-2-only infection in a dose-dependent manner (Fig. 4B). As expected, soluble LILRB1 ectodomain did not alter the rate of viral entry as this receptor functions by modulating the antiviral state of the cell rather than increasing DENV entry (SI Appendix, Fig. S6C and D). Likewise, reduced LILRB1 expression in THP-1.2S resulted in reduced DENV replication under ADE conditions (Fig. 4C), without altering the rate of viral entry (SI Appendix, Fig. S6E). The lack of any change in DENV replication with Fc γ RIIB expression also reinforces the notion that subneutralizing levels of antibody are insufficient to aggregate DENV to coligate Fc γ RIIB (9). Similar observations were made with knockdown of LILRB1 expression in another unrelated human myelogenous leukemia cell line, K562 (SI Appendix, Fig. S7).

Conversely, overexpression of LILRB1 in THP-1.2R resulted in increased DENV replication under ADE conditions (Fig. 4D).

Fig. 4. Coligation of LILRB1 is essential for ADE. (A) Binding of LILRB1 to whole DENV or DENV E protein ectodomain. (B) Plaque titers following DENV-2 or ADE infection in the presence of soluble LILRB1 ectodomain (2 μ M, 20 μ M, 200 μ M), 200 μ M BSA, or no protein control. (C) Plaque titers following DENV-2 or ADE infection after LILRB1 or Fc γ RIIB knockdown. Numbers below Western blot indicate levels of proteins relative to LAMP-1. (D) Plaque titers following DENV-2 or ADE infection in THP-1.2R transfected with empty vector or vector expressing LILRB1, mutant LILRB1 (LILRB1^{MUT}), or LILRB4. Numbers below Western blot indicate levels of proteins relative to LAMP-1. (E) Plaque titers following DENV-2-only and ADE infection of primary monocytes treated with sodium stibogluconate (SSG) or PBS control (dashed lines, shaded areas reflect SD). (F) Plaque titers following DENV-1–

As a control, we also overexpressed LILRB4, but this did not result in increased DENV replication. Critically, mutation of the four tyrosine residues in the ITIM tail to phenylalanine (*SI Appendix, Fig. S8*) abrogated the increased DENV replication (Fig. 4D). Taken collectively, these findings indicate that DENV coligates LILRB1 to inhibit Fc γ R-activated early ISG expression for ADE.

The mechanistic requirement for LILRB1 in ADE suggests that interfering with this pathway would abrogate ADE in primary monocytes. We studied CD14^{hi}CD16[–] inflammatory monocytes that express both Fc γ R and LILRB1 (*SI Appendix, Fig. S9 A and B*), which form the majority of the circulating monocytes (17). Indeed, pretreatment with sodium stibogluconate, a SHP-1 inhibitor resulted in a dose-dependent reduction in DENV-2 replication under ADE conditions (Fig. 4E), with no significant reduction in primary monocyte cytotoxicity (*SI Appendix, Fig. S9C*). Likewise, plaque titers following ADE infection of the other 3 DENV serotypes on primary monocytes obtained from different healthy donors were significantly lower in sodium stibogluconate treated cells compared with untreated cells (Fig. 4F). Pretreatment of primary monocytes derived from peripheral blood mononuclear cells (PBMCs) from 12 different healthy human volunteers with anti-LILRB1 antibodies also resulted in significantly reduced DENV replication compared with isotype antibodies (Fig. 4G).

Discussion

The ADE hypothesis has been widely used to explain the epidemiological association between secondary DENV infection and severe dengue (18, 19). However, entry through the activating Fc γ R pathway would pose no replicative benefit to DENV unless it is able to overcome the ITAM-Syk-STAT-1 signaling axis that leads to ISG induction (7, 13). The findings here thus indicate that coligation of LILRB1 is a critical first step for successful antibody-dependent DENV infection (*SI Appendix, Fig. S10*).

LILRB1 is expressed on monocytes, dendritic cells, and subsets of T and NK cells. Its natural function is to activate negative feedback mechanisms upon binding to major histocompatibility complex class I (MHC-I) molecules (20). Consequently, it is conceivable that viruses exploit this pathway to create an intracellular environment more favorable for replication. Besides dengue, human cytomegalovirus (HCMV) also binds LILRB1 through the glycoprotein UL-18 to trigger an inhibitory signaling pathway that limits antiviral effector functions (21, 22). Furthermore, increased LILRB1 expression in CD8⁺ effector T-cells is associated with reduced cytokine secretion and cytotoxicity in persistent HCMV and Epstein-Barr virus infections (22, 23). It would be interesting to test if LILRB1-mediated suppression of immune signaling is also exploited by other viruses.

Coligation of LILRB1 by DENV during antibody-dependent infection suggests that LILRB1 polymorphism may influence outcome of infection. Previous studies have shown that this gene is highly polymorphic (24) and can be alternatively spliced (25). However, a recent genome-wide association study did not reveal a significant association between LILRB1 and dengue shock syndrome (26); this is not surprising because, although LILRB1 activation is critical for initial replication with Fc γ R-mediated

–3, or –4-only and ADE infection of primary monocytes treated with SSG (0.138 mM) or PBS control. (G) Plaque titers in primary monocytes derived from PBMCs harvested from 12 healthy individuals and infected in vitro with either DENV-1 ($n = 3$), DENV-2 ($n = 3$), DENV-3 ($n = 3$), or DENV-4 ($n = 3$) opsonized with h4G2 antibodies at 72 hpi. PBMCs were either pretreated with polyclonal anti-LILRB1 antibody or isotype antibody control. Data are expressed as mean $\pm$ SD from three independent experiments. ** $P < 0.01$, * $P < 0.05$.

entry, multiple other host and viral factors contribute to eventual disease outcome.

Our findings also suggest that generation of antibodies to quaternary structure-dependent epitopes on DENV that block LILRB1 interaction can reduce ADE. That heterotypic antibodies can enhance dengue infection in FcγR-bearing cells represents a safety concern in the development of a dengue vaccine. Hence, a vaccine that can generate high-titer antibody that binds the quaternary structure-dependent epitopes on DENV to prevent LILRB1 ligation could reduce the risk of vaccine-induced ADE. Further studies would be needed to clarify this, although care must be taken in selecting a suitable in vivo model as the LILRB1 gene is deleted in laboratory strains of mice (27).

In conclusion, DENV coligates LILRB1 to down-regulate the activating FcγR-mediated early ISG expression for successful antibody-dependent infection.

Materials and Methods

Cells. THP-1.2R and THP-1.2S were subcloned from THP-1 by limiting dilution. Primary monocytes were isolated from healthy donors and cultured as described (9).

Viruses. DENV-1 (06K2402DK1), DENV-3 (05K863DK1), and DENV-4 (06K2270DK1) are clinical isolates from the EDEN study (28). DENV-2 (ST) is a clinical isolate from the Singapore General Hospital.

Virus Infection. Endotoxin-free (LAL Chromogenic Endotoxin Quantitation kit, Pierce) 3H5 and 4G2 chimeric human/mouse IgG1 antibodies were constructed as described (29). DENV was incubated with media, antibodies, or serum for 1 h at 37 °C before adding to cells at indicated MOI. Uptake was assessed using DiD and Alexa 488-labeled DENV as described (9, 30). For drug

assays, cells were pretreated with piceatannol (Sigma-Aldrich) or sodium stibogluconate (Santa Cruz Biotechnology) 6 h before infection. Cell viability was assessed using CellTiter 96 AQueous One Solution Cell Proliferation Assay (MTS, Promega) according to the manufacturer's protocol. Subsequently, virus replication was assessed using quantitative PCR at indicated time points and plaque assay at 72 h postinfection. Protein and protein phosphorylation levels were assessed using Western blots and analyzed with ImageJ.

Microarray Analysis. Following RNA extraction, microarray was performed at the Duke-NUS Genome Biology Core Facility. cRNAs were hybridized to Illumina Human HT-12 v4 Beadchips, according to manufacturer's instructions. Data analysis was performed using Partek software and normalized against GAPDH.

Competition with Soluble LILRB1 Ectodomain. The extracellular portion of LILRB1 was cloned into pCMV-XL5 (Origene) and transfected into HEK293T cells for protein expression. The expressed proteins were then purified and incubated with DENV-2 or h3H5-opsonized DENV-2 for 1 h at 37 °C before adding to THP-1.2S.

siRNA Transfection and Overexpression. siRNA transfections and overexpression were performed as described (9). siRNA targeting FcγRIIB (Qiagen), LILRB1, MAVS, IRF3, and TRIF (SABio) were used, and overexpression studies were performed with either empty plasmid, plasmid encoding LILRB1 or tyrosine mutant LILRB1, or LILRB4.

ACKNOWLEDGMENTS. We thank Soman Abraham for his constructive review of this work and Mei Fong Chan and Kenneth Goh for their technical assistance. This work was supported by the Singapore National Research Foundation under its Clinician-Scientist Award administered by the National Medical Research Council.

- Bhatt S, et al. (2013) The global distribution and burden of dengue. *Nature* 496(7446): 504–507.
- Halstead SB, O'Rourke EJ (1977) Dengue viruses and mononuclear phagocytes. I. Infection enhancement by non-neutralizing antibody. *J Exp Med* 146(1):201–217.
- Simmons CP, et al. (2007) Maternal antibody and viral factors in the pathogenesis of dengue virus in infants. *J Infect Dis* 196(3):416–424.
- Halstead SB (1988) Pathogenesis of dengue: Challenges to molecular biology. *Science* 239(4839):476–481.
- Halstead SB, O'Rourke EJ (1977) Antibody-enhanced dengue virus infection in primate leukocytes. *Nature* 265(5596):739–741.
- Libraty DH, et al. (2002) Differing influences of virus burden and immune activation on disease severity in secondary dengue-3 virus infections. *J Infect Dis* 185(9): 1213–1221.
- Dhodapkar KM, et al. (2007) Selective blockade of the inhibitory Fcγgamma receptor (FcγgammaRIIB) in human dendritic cells and monocytes induces a type I interferon response program. *J Exp Med* 204(6):1359–1369.
- Boonnak K, Slike BM, Donofrio GC, Marovich MA (2013) Human FcγRII cytoplasmic domains differentially influence antibody-mediated dengue virus infection. *J Immunol* 190(11):5659–5665.
- Chan KR, et al. (2011) Ligation of Fc gamma receptor IIB inhibits antibody-dependent enhancement of dengue virus infection. *Proc Natl Acad Sci USA* 108(30):12479–12484.
- Jiang D, et al. (2010) Identification of five interferon-induced cellular proteins that inhibit west nile virus and dengue virus infections. *J Virol* 84(16):8332–8341.
- Tsuchiya S, et al. (1980) Establishment and characterization of a human acute monocytic leukemia cell line (THP-1). *Int J Cancer* 26(2):171–176.
- Sheltzer JM, et al. (2011) Aneuploidy drives genomic instability in yeast. *Science* 333(6045):1026–1030.
- Tassiulas I, et al. (2004) Amplification of IFN-α-induced STAT1 activation and inflammatory function by Syk and ITAM-containing adaptors. *Nat Immunol* 5(11): 1181–1189.
- Steevels TA, Meyaard L (2011) Immune inhibitory receptors: Essential regulators of phagocyte function. *Eur J Immunol* 41(3):575–587.
- Fanger NA, et al. (1998) The MHC class I binding proteins LIR-1 and LIR-2 inhibit Fc receptor-mediated signaling in monocytes. *Eur J Immunol* 28(11):3423–3434.
- Scharenberg AM, Kinet JP (1996) The emerging field of receptor-mediated inhibitory signaling: SHP or SHIP? *Cell* 87(6):961–964.
- Passlick B, Flieger D, Ziegler-Heitbrock HW (1989) Identification and characterization of a novel monocyte subpopulation in human peripheral blood. *Blood* 74(7): 2527–2534.
- Halstead SB, Mahalingam S, Marovich MA, Ubol S, Mosser DM (2010) Intrinsic antibody-dependent enhancement of microbial infection in macrophages: Disease regulation by immune complexes. *Lancet Infect Dis* 10(10):712–722.
- Simmons CP, Farrar JJ, Nguyen V, Wills B (2012) Dengue. *N Engl J Med* 366(15): 1423–1432.
- Colonna M, et al. (1997) A common inhibitory receptor for major histocompatibility complex class I molecules on human lymphoid and myelomonocytic cells. *J Exp Med* 186(11):1809–1818.
- Yang Z, Bjorkman PJ (2008) Structure of UL18, a peptide-binding viral MHC mimic, bound to a host inhibitory receptor. *Proc Natl Acad Sci USA* 105(29):10095–10100.
- Cosman D, et al. (1997) A novel immunoglobulin superfamily receptor for cellular and viral MHC class I molecules. *Immunity* 7(2):273–282.
- Poon K, Montamat-Sicotte D, Cumberbatch N, McMichael AJ, Callan MF (2005) Expression of leukocyte immunoglobulin-like receptors and natural killer receptors on virus-specific CD8+ T cells during the evolution of Epstein-Barr virus-specific immune responses in vivo. *Viral Immunol* 18(3):513–522.
- Kuroki K, et al. (2005) Extensive polymorphisms of LILRB1 (ILT2, LIR1) and their association with HLA-DRB1 shared epitope negative rheumatoid arthritis. *Hum Mol Genet* 14(16):2469–2480.
- Jones DC, et al. (2009) Alternative mRNA splicing creates transcripts encoding soluble proteins from most LILR genes. *Eur J Immunol* 39(11):3195–3206.
- Khor CC, et al. (2011) Genome-wide association study identifies susceptibility loci for dengue shock syndrome at MICB and PLCE1. *Nat Genet* 43(11):1139–1141.
- Kubagawa H, Burrows PD, Cooper MD (1997) A novel pair of immunoglobulin-like receptors expressed by B cells and myeloid cells. *Proc Natl Acad Sci USA* 94(10): 5261–5266.
- Low JG, et al. (2006) Early Dengue infection and outcome study (EDEN) - study design and preliminary findings. *Ann Acad Med Singapore* 35(11):783–789.
- Hanson BJ, et al. (2006) Passive immunoprophylaxis and therapy with humanized monoclonal antibody specific for influenza A H5 hemagglutinin in mice. *Respir Res* 7:126.
- Zhang SL, Tan HC, Hanson BJ, Ooi EE (2010) A simple method for Alexa Fluor dye labelling of dengue virus. *J Virol Methods* 167(2):172–177.