Human Hematopoietic Reconstitution and HLA-Restricted Responses in Nonpermissive Alyphoid Mice

Malika Serra-Hassoun, Maryline Bourgine, Michele Boniotto, Julien Berges, Francina Langa, Marie-Louise Michel, Antonio A. Freitas and Sylvie Garcia

*J Immunol* published online 27 June 2014
http://www.jimmunol.org/content/early/2014/06/27/jimmunol.1400412

**Supplementary Material**
http://www.jimmunol.org/content/suppl/2014/06/27/jimmunol.1400412.DCSupplemental

**Subscription**
Information about subscribing to *The Journal of Immunology* is online at:
http://jimmunol.org/subscription

**Permissions**
Submit copyright permission requests at:
http://www.aai.org/About/Publications/JI/copyright.html

**Email Alerts**
Receive free email-alerts when new articles cite this article. Sign up at:
http://jimmunol.org/alerts
Human Hematopoietic Reconstitution and HLA-Restricted Responses in Nonpermissive Alymphoid Mice

Malika Serra-Hassoun, Maryline Bourgine, Michele Boniotto, Julien Berges, Francina Langa, Marie-Louise Michel, Antonio A. Freitas, and Sylvie Garcia

We generated a new humanized mouse model to study HLA-restricted immune responses. For this purpose, we created unique murine hosts by enforcing the expression of human SIRPα by murine phagocytes in murine MHC-deficient HLA-transgenic alymphoid hosts, an approach that allowed the immune reconstitution of nonpermissive mice following injection of human hematopoietic stem cells. We showed that these mouse/human chimeras were able to generate HLA-restricted responses to immunization. These new humanized mice may offer attractive models to study immune responses to human diseases, such as HIV and EBV infections, as well as to assay new vaccine strategies. *The Journal of Immunology, 2014, 193: 000–000.*

Over the last decades, many efforts have been made to establish human/mouse hematopoietic chimeras as models for “human” diseases, including HIV and EBV infections, as well as for designing vaccines. Although the absence of the IL-2R γ-chain (γc) significantly enhanced the immune reconstitution of alymphoid mice following human hematopoietic stem cell (HSC) engraftment, many strategies are ongoing to improve both qualitative and quantitative human immune systems in the murine hosts with the challenging goal of obtaining better adaptive T and B cell responses (1). These new strategies, which are primarily based on the deletion or introduction of new human genes (2, 3), are considerably impaired by the restricted use of BALB/c or NOD alymphoid mice, the only two strains reported to be permissive to engraftment by human HSCs (4). First, any non-NOD and non-BALB/c alymphoid mouse bearing interesting genetic features needs to be backcrossed to one of the “permissive” NOD or BALB/c backgrounds to support efficient human hematopoietic engraftment. Second, the direct introduction of new mutations in these mice is limited by the poor ability of both of these strains to be genetically manipulated.

In this article, we outline the generation of new non-BALB/c non-NOD immunodeficient recipient mice in which murine MHC was replaced by both HLA class I and HLA class II molecules and that were made permissive to human hematopoietic reconstitution through the enforced expression of the human SIRPα receptor by murine phagocytes. We show that these new humanized mice were able to generate HLA-restricted human CD8 T cell responses following immunization.

Materials and Methods

Mice

NOD SCID γc−/− (NSG) mice were purchased from The Jackson Laboratory, CH1-2 mice were generated by successive crossings of alymphoid RAG−/− γc−/− C57BL/6 mice, HLA-DR1−transgenic FVB/N mice (8), and HLA-A2−transgenic C57BL/6 mice (9) to finally obtain RAG−/− γc−/− C57BL/6 γc−/− β2m−/− I-A/B−/− DR1+ HLA-A2+ mice. The offspring were genotyped by PCR using tail DNA. Mice were housed in our animal facilities at Institut Pasteur in accordance with the Ethics Committee of Institut Pasteur Comité d’Ethique en Expérimentation Animale #89. All experiments were performed following procedures approved by the regional Comité d’EThique en Expérimentation Animale 59 Ethics Committee.

Fetal thymic organ cultures

Fetal thymic cultures (FTOCs), using murine thymus anlagen and human HSCs, were performed as previously described (10). Thymi were removed from day-14 embryos and irradiated at 300 rad using a [127Cs] gamma irradiator. Each thymic lobe was incubated in Terasaki wells for 2 d in 2 ml complete medium: RPMI 1640 supplemented with 10% heat-inactivated human serum, 5% FCS, 100 IU/ml penicillin, 100 μg/ml streptomycin, and 2 mM glutamine. A total of 10,000–30,000 CD34+ cells purified from cord blood (CB) was added to each well. The plates were immediately inverted to allow the formation of hanging drops and were incubated undisturbed in a humidified incubator (5% CO2 in air, 37°C). After 48 h, thymic lobes were transferred onto floating nucleopore filters (isopore membrane, 25 mm in diameter, pore size 8 μm; Millipore, Molsheim, France) in six-well plates in 2.5 ml complete medium and cultured for 28–35 d at 37°C in air supplemented with 5% CO2 with a weekly medium change. Recombinant human (rh)IL-2 (5 ng/ml), rhIL-7 (20 ng/ml), and rhSCF (50 ng/ml; Miltenyi Biotec) were included only during the first 48 h, mainly to prevent apoptosis of the human progenitors and early T cell progenitors. Cells were extracted from the different lobes by mechanical disruption, pooled, and analyzed by flow cytometry.

hSIRPα construction and transgenesis

The hSIRPα encoding cDNA [accession number NM_001040022, corresponding to sequence #2 described by Takenaka et al. (11)] was amplified by PCR from a previously described vector (kind gift from Dr. Axel Ullrich, Max-Planck-Institute for Biochemistry, Martinsried, Germany) using two primers designed to introduce the NheI/NotI restriction sites.

---

*Unité de Biologie des Populations Lymphocytaires, Département d’Immunologie, Institut Pasteur, 75724 Paris, France; †Centre National pour la Recherche Scientifique, Unité de Recherche Associée 1961, 75724 Paris, France; ‡Laboratoire de Pathogénèse des Virus de l’Hépatite B, Département de Virologie, Institut Pasteur, 75724 Paris, France; 3INSERM U845, 75724 Paris, France; 4Unit of Human Evolutionary Genetics, Department of Genome and Genetics, Institut Pasteur, 75724 Paris, France; and 5Centre d’Ingénierie Génétique Murine, Institut Pasteur, 75724 Paris, France

Current address: Institut Mondor de Recherche Biomédicale, INSERM U955, Hôpital Henri Mondor, Creteil, France.

Received for publication February 18, 2014. Accepted for publication May 25, 2014.

This work was supported by the National Agency for AIDS Research (Grant 08062-08006-09070), Agence Nationale de la Recherche-Institut Carnot Maladies Infectieuses, and Institut Pasteur.

Address correspondence and reprint requests to Dr. Sylvie Garcia, Unité de Biologie des Populations Lymphocytaires, Institut Pasteur, 25 Rue du Docteur Roux, 75724 Paris Cedex 15, France. E-mail address: sylvie.garcia@pasteur.fr

The online version of this article contains supplemental material.

Abbreviations used in this article: BM, bone marrow; γc, γ-chain; CB, cord blood; FTOC, fetal thymic organ culture; HBV, hepatitis B virus; HCV, hepatitis C virus; HP, hematopoietic progenitor; HSC, hematopoietic stem cell; NSG, NOD SCID γc−/−; rh, recombinant human; RT, room temperature.

Copyright © 2014 by The American Association of Immunologists, Inc. 0022-1767/14/$16.00
The Hnl1/N101 SIRPs sequence was inserted into a pGEL2B vector to replace a GFP sequence located downstream of a 7.2-kb fragment, which corresponds to the murine crm-s promoter (referred to as FIRE sequence; kind gift from Prof. David Hume, University of Edinburgh, Edinburgh, U.K.). Uncellular embryos from superovulated 3-wk-old CH1-2 mated females were microinjected with the 11-kb FIRE-SIRPs construct purified on agarose gel after enzymatic digestion of the vector (platform Centre d’Ingénierie Génétique Murine of Institut Pasteur). The resulting pups were engrafted by PCR using specific primers for hSIRPs: primer 5'-AGT GAG CTT CAC CTG CGA GT-3' and primer 5'-CAC GGG CTG TTG AGT AAC CT-3'. Seven of eleven pups tested positive for hSIRPs. All transgenic founders homogeneously expressed the hSIRpX receptor by flow cytometry (Fig. 1) and are referred to hereafter as CH1-2hSa mice.

Mice reconstitution
One- to two-day-old CH1-2 and CH1-2hSa neonates were irradiated with 400 rad using a [137]Cs gamma irradiator. Four hours after irradiation, the pups were injected in the liver with 50,000-100,000 hCD34+ cells (AbcellBio, Paris, France) in 30 µl PBS, using a 30-gauge needle (BD).

Human RBC in vivo injection
Human RBCs from the pellet obtained upon Ficoll-Hypaque gradient of human blood were resuspended at 4.10^6 cells/ml in PBS. They were labeled with 10 µM CFSE for 12 min at room temperature (RT) and washed in PBS containing 5% FCS. hCFSE-labeled RBCs were resuspended at 10^9 cells/ml in PBS, and 200 µl this solution was injected i.v. into CH1-2, CH1-2hSa, or NSG mice. The clearance of CFSE-hRBCs in host mice was analyzed by flow cytometry (FACS Fortessa; BD) or by immunohisto-fluorescence.

Immunohistofluorescence
Organs were washed in PBS and fixed overnight in 4% paraformaldehyde (Alfa-Aesar) in PBS under slow shaking at 4 °C. After a 1-d washing step in PBS, tissues were incubated in a bath of 30% sucrose (Sigma-Aldrich) in PBS for 3 h. Samples were embedded in O.C.T. compound (Prolabo), frozen in a bath of 2-methylenebutanol (Sigma-Aldrich), itself in a bath of liquid nitrogen, and stored at −70 °C. Frozen blocks were cut using a cryostat into 8-µm sections that were collected onto Superfrost Plus slides (VWR). Slides were blocked in solution (PBS 0.1% Triton X-100 and 10% Albumin from Bovine Serum; Sigma-Aldrich) for 1 h at RT and incubated overnight at 4°C with primary rat anti-mouse F4/80 mAb 1/50 dilution (Caltag Laboratories), followed by a second Cy3-conjugated goat anti-rat mAb 1/500 dilution (Invitrogen) for 1 h at RT. Finally, slides were incubated for 5 min at 20°C with DAPI (Sigma-Aldrich) and mounted with Fluorescent mounting media (FluoriGel; Axiovisio) or coverslips. Slides were examined with a Zeiss AxioImager M1 fluorescence microscope (Carl Zeiss) equipped with a charge-coupled device camera, and images were processed with Axiovision software (Carl Zeiss).

Immunizations
Five weeks after HSC engrafment, CH1-2hSa chimeras were bled, and the percentage of hCD45+ cells was determined. Mice that exhibited >20% hCD45+ were subjected to the vaccination protocol described in Fig. 5. Bilateral i.m. injection of cardiotoxin (12) (Latorox, France) was performed into the tibialis anterior muscles, followed by injection, at the same sites, of a mixture of 50 µg each plasmid DNAs coding for hepatitis B virus (HBV) core and envelope proteins in 100 µl PBS 5 d later (12). All i.m. injections were performed under anesthesia (ketamine/xylazine). Two weeks later, chimeras were boosted i.p. with a mix of 2 µg core and 2 µg envelope proteins (13) in 100 µl PBS containing 19% adjuvant (Alu-Gel-S; Serva, Heidelberg, Germany). As controls, some chimeras were injected twice with PBS after cardiotoxin injection. Two weeks after the last injection, chimeras were sacrificed, and spleens were removed.

Flow cytometric analyses
Single-cell suspensions from spleen, liver, and thymus from chimeras were washed in PBS containing 0.1% azide and 2% FCS (FACS buffer). They were incubated for 10 min with Fc block (anti-16/CD32; clone 2.4G2; BD Biosciences), washed, and stained for 10 min on ice with different combinations of anti-mouse H-2Dβ FITC (clone H2-Kb), I-Aβ6 PE (clone H11.14), CD11b PE-Cy7 (clone M1/70) (BD Biosciences), F4/80 Percp-Cy5.5 and CD11c Pacific Blue (clone N418), anti-mouse EpCam allophycocyanin (G8.8) (BioLegend), and GR1 FITC mAbs (clone RB6-865; eBioscience) or with anti-mouse or different combinations of anti-human HLA-DR1 V500 (clone G46.6), HLA-A2 PE (clone BB7.2), CD45 V500/PE-CF594 (clone HI30), CD3 Pacific Blue (clone UCHT1), CD4 allophycocyanin-Cy7/550/PE-CF594 (clone RPA-T4), CD8 PE/Percp-Cy5.5 (clone RPA-T8), CD19 Percp5.5 (clone HIB19), IgM allophycocyanin (clone G20-2127), IgD PE (clone IA6-2), CD16 FITC (clone 3G8), CD56 PE (clone Bi59), CD14 PE-Cy7 (clone M52E2), CD11c allophycocyanin (clone B-ly6), CD34 FITC (clone 581), CD117 PE-Cy7 (clone 104-D2), TCRαβ FITC (clone T109P-1A-31) (all from BD Biosciences) and CD172a allophycocyanin (clone 15-414; ebioscience) mAbs. Spleen cells from hematopoietic mice were incubated for 10 min with Fc block, as described in the previous section. Cells were stained for 10 min at RT in FACS buffer with a mix of three HBV-specific pentamers, HLA-A2-2env peptides (peptides 183-191 and 348-357) and HLA-A2-core peptide (peptides 18-27), all the three pentamers being conjugated with allophycocyanin (Proimmune, Oxford, U.K.). As controls, cells were incubated with hepatitis C virus (HCV)-specific pentamer HLA-A2 NS3 peptide (1073-1081). Cells were washed twice in FACS buffer and stained with 7-aminoactinomycin D 1/50 (Sigma) to exclude apoptotic cells, and anti-human CD45 PE/CF594, CD3 Pacific blue, CD4 V500, CD8 PE mAbs (for clones, see above) (BD Biosciences) in FACS buffer for 10 min on ice. In all cases, cells were washed in FACS buffer and processed on a FACS LSR Fortessa (BD). Data were analyzed using FlowJo software (TreeStar).

Results
HLA-transgenic murine MHC–deficient immunodeficient hosts induce better selection of human thymocytes ex vivo
To improve human lymphocyte function in humanized mice, we generated allogeneic RAGβ−/− γc−/− hosts, in which the mouse MHC was replaced by hHLA. In these conditions, better interactions between murine MHCα thymus epithelial cells and human hematopoietic cells were expected to improve thymus T cell selection. Furthermore, expression of hMHC should increase peripheral human T cell survival (14). Importantly, the exclusive education of human thymocytes by hHLA molecules in the absence of murine MHC should ensure strict “human” HLA–restricted responses to subsequent antigenic challenges. To devise such murine hosts, we interscrossed different available mutant strains: 129sv RAGβ−/− γc−/− allogeneic mice with C57BL/6 deficient mice for the HLA-A2 molecule (B2m−/−), C57BL/6 deficient mice for the β-chain of the MHC class II I-Aβ6 molecule (I-Aβ6−/−), C57BL/6 mice transgenic for the expression of the human MHC class I molecule HLA-A2 (HLA-A2*), and FVB/N mice transgenic for the expression of the hMHC class II molecule HLA-DR1 (HLA-DR1*) mice. We finally obtained RAGβ−/− γc−/− B2m−/− I-Aβ6−/− HLA-A2* HLA-DR1* mice, hereafter referred to as CH1-2 mice. Phenotypic analyses of both thymus and spleen from CH1-2 mice confirmed HLA-DR expression by EpCamα thymic epithelial cells and splenic CD11c+ dendritic cells (Fig. 1A) and HLA-A2 expression profile by thymic and splenic cells (Fig. 1B). We also confirmed the absence of the expression of both I-Aβ6 murine MHC class II (Fig. 1C) and H-2Dβ and H-2Kα murine MHC class I (Fig. 1D) in both thymic (upper panels) and splenic (lower panels) cells. Using FTOCs in which we seeded hCB CD34+ hematopoietic progenitors (HPSs) on CH1-2 and RAG−/− γc−/− thymus anlagen, we found that selection and maturation of human T cells were significantly improved when the thymus anlage expresses HLA, as shown by the increased generation of hCD3+ expressing cells (Supplemental Fig. 1).

Enforced expression of hSIRpα into CH1-2 hosts inhibits clearance of hRBCs in vivo and promotes CH1-2 host reconstitution by hHSCs
We then assessed the ability of CH1-2 hosts to be reconstituted by the human immune system upon their engraftment with hHPS. However, we were unable to detect any reconstitution of CH1-2hSa mice by hCD34+ cells (Fig. 2A, left panels). To promote immune reconstitution of CH1-2 hosts upon hHSC engraftment, we decided to enforce the expression of hSIRpα in CH1-2 phagocytes. For
this, expression of a cDNA coding for the hSIRPα sequence [2
as defined by Takenaka et al. (11)] was directed to mononuclear
phagocytes using a 7.2-kb sequence (referred as p7.2fms) con-
taining the proximal promoter of c-fms combined with the first
intron and the FIRE element of intron 2 (15). This construct was
reported to allow both consistent expression of the targeted gene
in the same locations as the endogenous gene and its appropriate
expression (15). This construct was injected into CH1-2 one-cell
oocytes. All founders, tested positive by PCR for the presence of
the hSIRPα-encoding cDNA, expressed the protein. As expected,
SIRPα was consistently coexpressed with murine CD11b, CD45,
GR1, CD11c, and F4/80 myeloid markers in the blood, spleen,
bone marrow (BM), and liver cells of CH1-2 mice (Fig. 2B) (15,
16). As controls for hSIRPα expression, CH1-2 mouse cells were
stained under the same conditions (Supplemental Fig. 2). CH1-2
mouse transgenic for hSIRPα were named CH1-2hSa. Impor-
tantly, xenogeneic CFSE-labeled hRBCs survived significantly
better when injected into transgenic CH1-2hSa mice than when in-
jected into their nontransgenic counterparts, as shown by a slower
clearance of CFSE-labeled hRBCs in the blood measured by flow
cytometry (Fig. 3A–C) and the decreased loss of CFSE+ hRBCs in
mice transgenic for hSIRPα (Fig. 3D–F), both similar to
those obtained in NSG hosts. Note that we found the same survival
rate of CFSE-labeled mouse RBCs after injection into the three
types of hosts, indicating that hRBC rejection in CH1-2 hosts was
due to their xenoreactivity (data not shown). We also were able to
visualize hRBCs engulfed by murine macrophages in CH1-2, but
not in CH1-2hSa, mice (Fig. 3G). These results confirmed the
functionality of the hSIRPα transgene in vivo.

We engrafted hCD34+ CB HPs into irradiated neonatal CH1-
2hSa mice heterozygous for hSIRPα. Strikingly, we found that
the presence of hCD45+ cells in the blood of engrafted mice
was associated with the expression of hSIRPα by the host cells
(Fig. 2A, right panels). This result was observed for all of the
hCD45+ cell samples used to assess the reconstitution of CH1-
2hSa hosts. In addition, the percentage of hCD45+ cells in
the blood of CH1-2hSa chimera mice was comparable to that
obtained when NSG mice were used as hosts (Fig. 4D). Human
immune peripheral reconstitution revealed the presence of CD4+ and
CD8+ T and IgM+ IgD+ B lymphocytes in the spleen of CH1-
2hSa chimera as in NSG chimera (Fig. 4A, 4B), although with a
higher B/T cell ratio in CH1-2hSa mice compared with NSG
hosts (Fig. 4E). hNK cells also were detected at a higher pro-
portion in CH1-2hSa chimeras compared with NSG chimeras
(Fig. 4C–E), consistent with the reported role of SIRPα in NK
cell homeostasis (17, 18). As controls, the same subsets were ana-
yzed in CB cells. As expected, human T cell development was
normal, as supported by the presence of the different cellular sub-
populations in the thymus of CH1-2hSa chimeras (Fig. 5A, 5B).
hNK cells also were found in the thymus of CH1-2hSa chimeras
(Fig. 5C). Similarly, both immature IgD− IgM+ human B cells
and, to a lesser extent, mature IgD+ IgM+ human B cells (Fig. 5D,
5E), as well as human NK cells (Fig. 5F), human myeloid dendritic
cells and human monocytes/macrophages (Fig. 5G), and hCD34+
populations in the thymus of CH1-2hSa chimeras (Fig. 5A, 5B).

Induction of HLA-restricted human T cell response in hSIRPα
HLA-transgenic chimeras

One of the expected validations of the new CH1-2hSa chimera was
the induction of HLA-A2–restricted hCD8+ T cell responses. We
immunized CH1-2hSa chimeras with injections of two plasmids
encoding HBV envelope and capsid, followed by a mixture of the
corresponding purified recombinant proteins (Fig. 6A). Two
weeks after the last immunization, an HLA-A2–restricted anti-
HBV splenic hCD8+ T cell response was detected directly using
specific MHC pentamers in all kinds of samples
(Fig. 6B) (19). HLA-A2–restricted HBV-specific pentamer
+ CD8+ T cells were detected only in immunized
CH1-2hSa chimeras and not in nonimmunized chimeras or in the
original HLA-A2+ CB cells (Fig. 6C). As controls for HLA-A2
pentamer stainings, no positive cells were observed using irrele-
vant HCV-specific HLA-A2 pentamers in all kinds of samples
(Fig. 6C). The magnitude of the response varied from 1.2 to 2.77%
of the hCD3+CD8+ T cells in the immunized CH1-2hSa chimeras (Fig. 6D,
upper panel). No response was obtained if the CD34+ cells
used to engraft the immunized CH1-2hSa host were HLA2−
(Fig. 6D, lower panel).

Discussion

Humanized mice are promising murine models to study human
diseases. In particular, human immune chimeras resulting from
hHSC engraftment (HIS mice) are the models of choice to study
human infections or immunopathogenesis and/or to devise new
vaccination protocols. Although humanized mice have been suc-
cessfully infected with viruses, such as HIV, EBV, or dengue, the
induction of an HLA-restricted human T cell response, as well
as specific switched IgG production, is limited (1). Some groups
reported that the introduction of HLA expression by the murine hosts greatly improved human responses in HIS mice (20–23). Although murine MHC probably plays a major role in thymic selection of human T cells in the chimeras (20, 24), none of the previous studies could exclude the generation of a mouse-restricted human T cell response, precluding the use of these chimeras as preclinical models for vaccine design or immune responses to infections. Therefore, we generated murine hosts in which murine MHC molecules were replaced by HLA molecules to ensure strict education of the human thymocytes by HLA molecules. For this, we took advantage of several existing genetically modified mice to create immune-deficient mice. Although we could not achieve any human immune reconstitution in the first version of the murine hosts, we showed that murine HLA-expressing thymus anlages supported maturation of human thymocytes better than did non–HLA-expressing thymic anlages, arguing for a positive role for HLA-expressing murine thymic cells in human T cell development.

SIRPα (also known as SHPS1, MYD1, or CD172a) is a transmembrane protein containing three Ig-like domains in its extracellular region and putative tyrosine phosphorylation sites in its cytoplasmic region (25, 26). Although its ligand CD47 is ubiquitously expressed, the expression of SIRPα is mainly restricted to neurons and to phagocytes, macrophages, dendritic cells, and neutrophils. One of the main roles of SIRPα/CD47 interactions is the regulation of macrophage-mediated phagocytosis (27, 28). In addition, SIRPα was shown to express different alleles in both human and mice populations (11). Consistent with this function, the ability of NOD immune-deficient hosts to promote immune reconstitution upon hHSC engraftment was attributed to better xenogeneic hCD47/mouse SIRPα recognition, leading to the inhibition of human donor cell destruction by murine phagocytes both in vitro and in vivo (11, 29).

We chose to enforce hSIRPα expression in the phagocytes of CH1-2 mice by taking advantage of the well-documented properties of the three strains used to generate them: the vigorous reproduction capacities, with big and regular litters, and the large and prominent pronuclei facilitating DNA microinjection, which are characteristic of FVB/N mice (30); the high rate of homologous recombination and of germ-line transmission for the embryonic stem cells, which are characteristic of 129/SvJ mice (31, 32); and the high resistance to tumor formation, long life, and good reproduction rate, which are characteristic of C57BL/6 mice. To direct the expression of hSIRPα in the CH1-2 phagocytes, we used a previously described promoter of the c-fms gene containing the FIRE region, allowing the targeted expression of many phagocytes in the transgenic mice, and of monocytes, macrophages, neutrophils, and other unidentified GR1 cells in different organs of the mice. In line with what was described upon introduction of the NOD SIRPα by intercross in alymphoid C57BL/6 mice (29), our results demonstrate that the expression of hSIRPα only in phagocytes of murine nonpermissive alymphoid hosts is

![Figure 2](http://www.jimmunol.org/)

FIGURE 2. Expression of hSIRPα by murine phagocytes induces hematopoietic development of CH1-2hSa hosts upon hHSC engraftment. (A) CH1-2 and heterozygous CH1-2hSa neonates were engrafted with hHSCs, and blood and spleen cells were analyzed 7 wk later by flow cytometry. Dot plots of hCD45 versus murine CD45 expression (upper panels) and hCD45 versus hSIRPα expression (lower panels) by blood cells from CH1-2 (left panels) and CH1-2hSa (right panels) chimeras. (B) Dot plots of the coexpression profiles of the hSIRPα transgene and murine CD11b, CD45, GR1, CD11c, and F4/80 analyzed by flow cytometry on blood, spleen, BM, and liver cells of CH1-2hSa alymphoid mice. The data are representative of six mice.
sufficient to allow hHSC engraftment. The approach that we report offers several advantages: the murine SIRPα of the hosts was untouched, precluding any unwanted host cell destruction and keeping the physiology of the hosts; the construct targets virtually all phagocytes, ensures reliable expression, and highly increases transgenesis efficiency; and transgenesis may be suitable for many strains reported as “nonpermissive” and is relevant when many genetic modifications of the hosts are involved (as for CH1-2 recipients). The successful introduction of human growth factors to improve human cell development, differentiation, and maintenance was reported (reviewed in Ref. 1). In addition to enforced expression of such factors in CH1-2hSa mice, the introduction of HLA haplotypes other than HLA-A2 and HLA-DR1, originally chosen for their relatively high frequencies in western Europe.
(9–21% and 30–50%, respectively; http://www.allelefrequencies.net), should increase the level of positively selected human T cells in murine host thymus and their peripheral survival, as well as favor the use of other matched human progenitors. The level of reconstitution of transgenic SIRP$\alpha$ mice was comparable to that of NSG mice, the current gold standard of humanized mice. Interestingly, the expression of human or NOD SIRP$\alpha$ in murine hosts was shown to improve hNK and T cell numbers compared with the original BALB/c hosts (17, 18). In addition to their immune reconstitution, which is comparable to that in previously described humanized mice, CH1-2hSa mice offer higher radioresistance to radiation than do BALB/c or SCID strains, which are known to be sensitive to it (33, 34). This property is expected to impact both the fitness and survival of the chimera.

Using these new recipients, we were able to generate functional immune chimeras that were able to mount an HLA-restricted human response that we could detect in a direct manner by multimer HLA peptide technology, an approach used in only one study (35) describing the use of HLA-transgenic hosts. Of note, we

FIGURE 4. Multilineage reconstitution of CH1-2hSa hosts upon hHSC engraftment. (A–C) Spleen cells from 5-mo engrafted CH1-2hSa (top panels) and NSG (middle panels) chimeras were analyzed by flow cytometry. CB cells were analyzed as controls (bottom panels). Dot plots of CD4 versus CD8 expression (after gating on hCD3$^+$ CD45$^+$ cells) (A), IgM versus IgD expression (after gating on hCD19$^+$ CD45$^+$ cells) (B), and CD56 versus CD16 expression (after gating on CD3$^+$ CD45$^+$ cells) (C). (D) Percentages of blood hCD45$^+$ cells in individual CH1-2hSa and NSG chimeras 4–5 mo after engraftment by hHSCs. (E) Bar graph showing the distribution of T, B, NK, and other human subsets among hCD45$^+$ spleen cells in CH1-2hSa and NSG chimeras 4–5 mo after engraftment. Dot plots are representative of 16 CH1-2hSa chimeras, 5 NSG chimeras, and 8 CB cells. Graphs are based on the mean of the 16 CH1-2hSa chimeras and 5 NSG chimeras.

FIGURE 5. Thymic and BM reconstitution of CH1-2hSa chimeras by human cells. CH1-2hSa chimeras were sacrificed 5 mo after hHSC engraftment, and thymus and BM human cells were analyzed by flow cytometry. (A) Dot plots show CD4 versus CD8 expression by hCD45$^+$ cells in the thymus of CH1-2hSa chimeras. The percentages of CD4$^+$ CD8$^+$ (DP), CD4$^+$ CD8$^-$ (SP CD4$^+$), and CD8$^+$ CD4$^-$ (SP CD8$^+$) human thymocytes are indicated. (B) Graph shows TCRab expression by DP, SP CD4$^+$, and SP CD8$^+$ hCD45$^+$ thymocytes. (C) Dot plot shows CD56 versus CD16 expression by hCD45$^+$ CD3$^-$ NK cells in the thymus of CH1-2hSa chimeras. Data are representative of five mice. (D) Dot plot shows CD19 versus hCD45 expression (percentage is indicated). (E) Dot plot shows IgM and IgD expression on CD19$^+$ hCD45$^+$ cells from the dot plot in (D). (F) Dot plot of CD16 versus CD56 after gating on CD45$^+$ CD3$^-$ human cells. (G) Dot plot of CD14 versus CD11c expression after gating on hCD45$^+$ cells. (H) Dot plot of CD34 versus CD117 (cKit) expression after gating on hCD45$^+$ cells. Dot plots are representative of five mice.
did not observe any response when the haplotype of the donor CD34+ cells was different from the host HLA-A2 transgene (data not shown). This latter observation indicates that either the human T cells selected in the thymus by HLA-A2+ mouse epithelial cells could not cooperate with the HLA-A2+ human cells or with the HLA-A2+ mouse APCs to induce a response or, alternatively, that the human T cells were mainly selected by HLA-A2+ human hematopoietic-derived cells and were not able to recognize HLA-A2/peptide complexes. Cell cooperation involving interactions between MHC class I and class II–peptide complexes on APCs and B cells with TCR on CD4 and CD8 T cells was shown to be required for IgG switching during B cell responses (36), as well as for memory CD8 T cell generation (37). These interactions rely on the matching between MHC involved during thymic selection for both CD4 and CD8 T cells and APCs (professional dendritic cells and B cells, as well as target cells) at the periphery. Although we did not directly address these issues in the present study, CH1-2hSa mice were designed to fulfill these criteria and to support optimized immune responses. Although the HLA haplotype of the transplanted hHSCs usually matched that of the transgenes expressed by the reported HLA-transgenic murine recipients (20–23), one recent report showed an Ag-specific response by hCD8 T cells expressing an HLA allele different to that expressed by the murine hosts (35). This still raises the possibility that, in some cases, and in contrast to what we observed, murine HLA–expressing peripheral cells could present Ag to activate human T cells.

In conclusion, this method allowed us to generate unique aliphoid hosts, in which the development and function of human T cells occur in a strict human context, through the expression of HLA as exclusive MHC molecules. Therefore, CH1-2hSa chimeras may represent powerful models to study immune responses associated with infections, cancers, or vaccines, as well as autoimmune diseases. In addition, the introduction of hSIRPa may switch any existing non-NSG non-BALB/c aliphoid mice bearing mutations/alleles of interest and mimicking human diseases (38) into hosts suitable for human reconstitution, opening avenues for generating new humanized mouse models.

Acknowledgments
We thank Dr. Axel Ullrich and Prof. David Hume for providing the hSIRPa and c-fms vectors, respectively. We also thank Dr. Cyrille Ramond for excellent help with FTOC experiments.

Disclosures
An international patent # PCETP 2013056443 A1 concerning the CH1-2hSa mice is pending. M.S.-H. and S.G. are listed in the patent.