(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
14 August 2014 (14.08.2014)

WIPO | PCT

(10) International Publication Number
WO 2014/121840 A1

(51) **International Patent Classification**:
A61K 39/395 (2006.01) *C12N 5/0784* (2010.01)

(21) **International Application Number**:
PCT/EP2013/052485

(22) **International Filing Date**:
7 February 2013 (07.02.2013)

(25) **Filing Language**: English

(26) **Publication Language**: English

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(81) **Designated States** *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) **Designated States** *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:

— *with international search report (Art. 21(3))*

— *with sequence listing part of description (Rule 5.2(a))*

WO 2014/121840 A1

(54) **Title**: INDUCED DENDRITIC CELLS AND USES THEREOF

(57) **Abstract**: The present invention relates to cells engineered to express at least one cytokine and at least one antigen which induces the self differentiation of dendritic cell (DC) progenitor cells into functional antigen-presenting induced DC (iDC). Moreover, therapeutic uses of said iDC for regenerating the immune system after transplantation of hematopoietic stem cells are disclosed. Said iDC are also useful for generating mice with a functional humanized immune system which can be used as animal models for the study of the human adaptive immune responses.

Induced dendritic cells and uses thereof

The present invention relates to cells engineered to express at least one cytokine and at least one antigen which induces the self differentiation of dendritic cell (DC) progenitor cells into functional antigen-presenting induced DC (iDC). Moreover, therapeutic uses of said iDC for regenerating the immune system after transplantation of hematopoietic stem cells are disclosed. Said iDC are also useful for generating mice with a functional humanized immune system which can be used as animal models for the study of the human adaptive immune responses.

Background of the Invention

Hematopoietic stem cell transplantation (HSCT) has become the standard therapeutic approach for diverse malignant hematopoietic disorders, accounting with high rates of clinical success for chronic (80%) and acute myeloid leukemia (65%) due to long term hematopoietic engraftment and graft versus leukemia effects of the donor graft. However, post-HSCT subjects suffer from profound cellular immunodeficiency the first 100 days post-transplantation and reach full T cell reconstitution only after 1 year post-HSCT. Longer periods of cell immune deficiency are associated with subsequent increase of risk for opportunistic viral and fungal infections and relapse (up to 40%) (Seggewiss, R., and H. Einsele, 2010, "Immune reconstitution after allogeneic transplantation and expanding options for immunomodulation: an update." *Blood* 115: 3861-3868; Roncarolo, M. G. et al., 2011, "Clinical tolerance in allogeneic hematopoietic stem cell transplantation." *Immunological reviews* 241: 145-163; Mori, T., and J. Kato, 2010, "Cytomegalovirus infection/disease after hematopoietic stem cell transplantation" *International journal of hematology* 91: 588-595). Thus, strategies to accelerate the recovery of the lymphocyte pool with a broad repertoire of T cell responses and induce optimal viral immunity in post-HSCT transplanted subjects are required.

Novel advance therapeutic approaches to induce immune reconstitution in immunodeficient hosts based on passive and active immunization have been developed over the past decade. Yet, suitable *in vivo* experimental models to address efficacy and biosafety of such therapies are still under development. In order to experimentally recapitulate human immune reconstitution after HSCT in vivo, CD34+ human hematopoietic stem cells (HSC) are transplanted into diverse immunodeficient mouse strains lacking the common interleukin-2 receptor gamma chain (IL2Rγ) (NOD-Rag1nullIL2Rγ^{null}-NRG, NOD/LtSz-scid/IL2Rγ^{null}-NSG, or NOD/SCID/IL2Rγ^{null}-NOG) after sublethal total body irradiation (TBI), resulting in reconstitution of human hematopoietic lineages 8 to 10 weeks after CD34+ cell transfer (Ishikawa, F., et al., 2005, "Development of functional human blood and immune systems in NOD/SCID/IL2 receptor γ chainnull mice." *Blood* 106: 1565-1573). Importantly, regardless the source of HSCs and the method of cell transplantation, humanized mice

displayed suboptimal levels of lymphocyte reconstitution, lack of antigen specific cellular and humoral responses and overall anergy (Lepus, C. M. et al., 2009, "Comparison of Human Fetal Liver, Umbilical Cord Blood, and Adult Blood Hematopoietic Stem Cell Engraftment in NOD-scid/γc−/−, Balb/c-Rag1−/−γc−/−, and C.B-17-scid/bg Immunodeficient Mice." *Human immunology* 70: 790-802; Andre, M. C. et al, 2010, "Long-term human CD34+ stem cell-engrafted nonobese diabetic/SCID/IL-2R gamma(null) mice show impaired CD8+ T cell maintenance and a functional arrest of immature NK cells." *J Immunol* 185: 2710-2720). Factors that determine the inefficient lymphatic development in humanized mice include the absence of human histocompatibility molecules, impaired thymic function and poor human cytokine environment.

Attempts to solve this problem included delivery of cytokines (O'Connell, R. M. et al., 2010, "Lentiviral Vector Delivery of Human Interleukin-7 (hIL-7) to Human Immune System (HIS) Mice Expands T Lymphocyte Populations." *PLoS One* 5; Chen, Q. et al., 2009, "Expression of human cytokines dramatically improves reconstitution of specific human-blood lineage cells in humanized mice." *Proc Natl Acad Sci U S A* 106: 21783-21788), transplantation of fetal lymphatic tissue along with HPCs (Hu, Z., and Y. G. Yang, 2012, "Human lymphohematopoietic reconstitution and immune function in immunodeficient mice receiving cotransplantation of human thymic tissue and CD34+ cells" *Cell Mol Immunol* 9: 232-236; Biswas, S. et al., 2011, "Humoral immune responses in humanized BLT mice immunized with West Nile virus and HIV-1 envelope proteins are largely mediated via human CD5+ B cells." *Immunology* 134: 419-433) and the use of transgenic strains constitutively expressing the major histocompatibility molecules (MHC) class I (Shultz, L. D. et al, 2010, "Generation of functional human T-cell subsets with HLA-restricted immune responses in HLA class I expressing NOD/SCID/IL2rγnull humanized mice." *Proc Natl Acad Sci U S A* 107: 13022-13027) and HLA class II (Danner, R. et al., 2011, "Expression of HLA Class II Molecules in Humanized NOD.Rag1KO.IL2RgcKO Mice Is Critical for Development and Function of Human T and B Cells" *PLoS One* 6) or critical hematopoietic cytokines (Willinger, T. et al., 2011, "Human IL-3/GM-CSF knock-in mice support human alveolar macrophage development and human immune responses in the lung" *Proc Natl Acad Sci U S A* 108: 2390-2395) have been recently described. These strategies allowed a limited improvement in B and T cell responses against human viral challenges. Importantly, only few reports have described the presence of reconstituted lymphatic structures in HSC-transplanted mice (Singh, M. et al., 2012, "An Improved Protocol for Efficient Engraftment in NOD/LTSZ-SCIDIL-2RγNULL Mice Allows HIV Replication and Development of Anti-HIV Immune Responses" *PLoS One* 7; Marodon, G. et al., 2009, "High diversity of the immune repertoire in humanized NOD.SCID.gamma c-/- mice" *European journal of immunology* 39: 2136-2145; Sun, Z. et al., 2007, "Intrarectal transmission, systemic infection, and CD4+ T cell depletion in humanized mice infected with HIV-1" *J Exp Med* 204: 705-714). Although high quality CD34+ HPC from human cord blood or fetal liver were used, thereby reaching high rates of human cell engraftment and reasonable levels of human lymphatic cells, lymph nodes (LN) were barely observed. These data

suggest that lack of lymphatic organ regeneration could be playing an important role in the poor lymphoid cell reconstitution observed in humanized mouse models.

DC play a central role in the induction of adaptive immune responses. Importantly, DC trigger the regeneration and remodeling of tertiary lymphatic structures and play a fundamental role in maintaining the function of LN during active immune responses.

Using lentivirus (LV)-mediated gene transfer, we have developed a method to generate highly viable and potent DCs for cancer immunotherapy (Pincha, M. et al., 2011, "Lentiviral vectors for induction of self-differentiation and conditional ablation of dendritic cells" *Gene therapy* 18: 750-764; Koya, R. C. et al., 2007 "Lentiviral vector-mediated autonomous differentiation of mouse bone marrow cells into immunologically potent dendritic cell vaccines" *Molecular Therapy* 15: 971-980". LV-induced DC showed high levels of engraftment and potent capacity to stimulate antigen specific responses and protect against melanoma *in vivo*. Recently, we demonstrated that integrase-defective (ID) LV gene delivery of human granulocyte-macrophage colony stimulation factor (GM-CSF) and interferon (IFN)-α into human monocytes resulted in autonomous differentiated and highly viable dendritic cells (Daenthanasanmak, A. et al., 2012, "Integrase-defective lentiviral vectors encoding cytokines induce differentiation of human dendritic cells and stimulate multivalent immune responses in vitro and in vivo" *Vaccine* 30: 5118-5131).

Thus, the problem underlying the present invention can be viewed as the provision of means and methods which improve the regeneration of the immune system after transplantation of hematopoietic stem cells.

The problem is solved by the embodiments described in the claims and the description below.

Summary of the Invention

In one aspect, the present invention relates to an induced dendritic cell (iDC) engineered to express

a) at least one cytokine which induces the self-differentiation of human DC progenitor cells into DCs; and

b) at least one antigen;

for use as a medicament.

In another aspect, the present invention relates to an induced dendritic cell (iDC) engineered to express

a) at least one cytokine which induces the self-differentiation of human DC progenitor cells into DCs; and

b) at least one antigen;

for use in the regeneration of the immune system of an immunodeficient subject following transplantation of HSC.

In yet another aspect, the present invention relates to an iDC engineered to express

a) at least one cytokine which induces the self-differentiation of human DC progenitor cells into DCs; and

b) at least one antigen;

for use as a medicament for the treatment of cancer which spreads lymphatically or a disease caused by a lymphotrophic pathogen.

In yet another aspect, the present invention relates to a method for regenerating an immune system in an immunodeficient subject comprising the steps of

a) transplanting hematopoietic stem cells to the subject; and

b) administering to the subject an iDC engineered to express at least one antigen and at least one cytokine which induces the self-differentiation of DC progenitor cells into DCs .

In yet another aspect, the present invention relates to an animal model with a functional xenogeneic immune system produced by the method of the present invention.

In yet another aspect, the present invention relates to the use of a mouse with a humanized immune system produced by the method of the present invention for the study of the human immune system or for the testing of drugs, implants or devices for their use in humans.

In yet another aspect, the present invention relates to an iDC comprising at least one integrase-defective lentiviral vector, wherein said vector mediates expression of

a) at least one cytokine which induces the self-differentiation of human DC progenitor cells into DCs; and

b) at least one antigen.

In yet another embodiment, the present invention relates to a method for generating iDCs comprising the steps of

a) isolating progenitor cells from a sample derived from a suitable donor;

b) engineering the cells to achieve expression of at least one cytokine which induces the self-differentiation of human dendritic cell (DC) progenitor cells into DCs and at least one antigen.

Detailed Description of the Invention

Before the present invention is described in detail below, it is to be understood that this invention is not limited to the particular methodology, protocols and reagents described herein as these may vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only, and is not intended to limit the scope of the present invention which will be limited only by the appended claims. Unless defined otherwise, all technical and scientific terms used herein have the same meanings as commonly understood by one of ordinary skill in the art. Preferably, the terms used herein are defined as described in "A multilingual glossary of biotechnological terms: (IUPAC Recommendations)", Leuenberger, H.G.W, Nagel, B. and Kölbl, H. eds. (1995), Helvetica Chimica Acta, CH-4010 Basel, Switzerland).

Throughout this specification and the claims which follow, unless the context requires otherwise, the word "comprise", and variations such as "comprises" and "comprising", will be understood to imply the inclusion of a stated integer or step or group of integers or steps but not the exclusion of any other integer or step or group of integer or step.

Several documents are cited throughout the text of this specification. Each of the documents cited herein (including all patents, patent applications, scientific publications, manufacturer's specifications, instructions, GenBank Accession Number sequence submissions etc.), whether supra or infra, is hereby incorporated by reference in its entirety. Nothing herein is to be construed as an admission that the invention is not entitled to antedate such disclosure by virtue of prior invention.

Description of the iDC of the present invention

The present invention relates to an induced dendritic cell (iDC) engineered to express

a) at least one cytokine which induces the self-differentiation of human dendritic cell (DC) progenitor cells into DCs; and

b) at least one antigen.

An induced dendritic cell (iDC) useful for the methods and uses described further below in this application is a dendritic cell which is induced to self-differentiate by the expression of at least one cytokine and at least one antigen.

The term "self-differentiation" of a dendritic cell refers the development of a dendritic cell from a suitable progenitor cell which is stimulated by endogenous expression of at least one cytokine and at least one antigen. The term "self-differentiation" does not exclude the addition of cytokines to the growth medium during the process of differentiation. However, the endogenous expression of at least one cytokine, which induces the self-differentiation of DC progenitors into DCs is required for this process, i.e. self-differentiation does not take place if the iDC is not engineered to achieve expression of the at least one cytokine. The engineering of iDCs is, preferably, a process which is performed *in vitro*.

In a preferred embodiment, all cytokines and antigens required for the differentiation of a progenitor cell into a dendritic cell are endogenously expressed by the progenitor cell and the resulting iDC.

Any cell which can differentiate into a dendritic cell is a suitable progenitor for an iDC according to the present invention. Thus, the term "progenitor cell" encompasses pluripotent stem cells and hematopietic stem cells. Preferably, the progenitor cell to the dendritic cell is a monocyte, more preferably a peripheral blood monocyte. Preferably, said monocyte is characterized by presence of CD14 on its cell surface. Human monocytes that are $CD14^+CD16^-$ are considered *classical* monocytes, whereas $CD14^+CD16^+$ cells are *non-classical* monocytes. Both types of monocytes are preferred. Monocytes express receptors for: GM-CSF, M-CSF, G-CSF and chemokine receptors CCR1, CCR2 and CCR5. Therefore, a progenitor cell is defined by the expression of at least one gene selected from the group consisting of CD14, CD16, GM-CSF, M-CSF, G-CSF, CCR1, CCR2 and CCR5. More preferably, the progenitor cell is characterized by the expression of at least 2, 3 or 4 of the aforementioned genes. Moreover, the progenitor does, preferably, not express CD3, CD19, CD20 and CD56 as these are markers for T-lymphocytes, B-lymphocytes and natural killer cells.

The progenitor cell to the iDC is, preferably, derived from an organism selected from the group consisting of primate, rodent, cat dog, pig, cow and sheep. The primate is, preferably, a human, a chimpanzee or a macaque, most preferably a human. The rodent is, preferably, a mouse or rat, most preferably a mouse.

Preferably, a suitable progenitor cell is derived from blood (peripheral or umbilical cord blood) by selecting cells which express CD14 on their surface.

The term "engineering" is used to refer to a process, which leads to the expression of one or more cytokines and/or one or more antigens, which are not naturally expressed by the DC progenitor or the DC or not naturally expressed at the level. In a preferred embodiment the cell is engineered by the introduction of a vector comprising nucleic acid sequences encoding the one or more cytokines and/or the one or more antigens. It is possible that these encoding nucleic acids sequences are comprised in one vector or on separate vectors, e.g. nucleic acid sequences encoding one or more cytokines are comprised on one vector and nucleic acid sequences encoding one or more antigens are comprised on another vector. In another preferred embodiment the DC progenitor or DC is engineered to express a cytokine and/or antigen, which is naturally encoded in the human genome but the expression of which is usually silenced or repressed in DC progenitors or DCs. This may be achieved by art known methods including the introduction of promoter and/or enhancer sequences in the vicinity of the gene encoding the respective cytokine and/or antigen. If promoters are introduced, it is preferred to introduce them upstream of the coding region of the respective gene in a way that these promoters direct the expression of the endogenous cytokine and/or antigen. Preferably, strong constitutive promoters are introduced like viral promoters, e.g. CMV immediate early promoter or SV40 promoter or promoters of housekeeping genes. In an alternative embodiment regulable promoter systems are used, which allow adaptation of the expression and of the expression level of the cytokine and/or antigen as required. Such regulatable promoter systems comprise, e.g. the Tet^{on}, Tet^{off} or lac repressor system. Again different promoter systems may be used for the cytokines and the antigens, e.g. regulatable promoter for the cytokines and constitutive promoter for the antigen. The approach of using a vector can be combined with the approach of introducing a heterologous promoter/enhancer, e.g. the cell can be engineered to express one or more cytokines which are encoded in the human genome by introduction of a suitable promoter/enhancer into the vicinity of the respective cytokine gene and can be transfected with a vector comprising a nucleic acid sequence encoding one or more antigens.

The term "endogenous expression" of an antigen or a cytokine refers to production of said molecules by the dendritic cell itself and/or its progenitor cell. Said endogenous expression is either mediated by up-regulation of a cellular gene encoding said cytokine or antigen or by recombinant expression of the cytokine or antigen as outlined above.

Up-regulation of a cellular gene is, preferably, achieved by transcriptional activation of a cellular gene under the control of a tissue-specific, inducible or constitutive promoter. It is also preferred to transfect the cell with a nucleic acid that can activate transcription of a specific gene or a nucleic acid encoding a regulatory protein, such as a transcription factor which – if it is expressed in the cell –

induces transcription of the endogenous cytokine gene. However, it is generally easier –and therefore preferred – to use recombinant expression the cytokine or antigen.

The term "recombinant expression" refers to the expression of a gene in the dendritic cell or its progenitor cell, wherein the gene is comprised by a nucleic acid molecule introduced into the cell. Methods for introducing foreign nucleic acid molecules into eukaryotic cells are well known in the art. In principle, any such method can be used as long as it results in the desired expression of the at least one cytokine and the at least one antigen by the cell without impairing the development or function of the dendritic cell.

Thus, in a preferred embodiment of the present invention the engineering of the iDC to induce expression of at least one cytokine which induces the self-differentiation of human dendritic cell (DC) progenitor cells into DCs and at least one antigen is the introduction of a vector encoding said proteins into the DC or its progenitor cell.

Vectors

Preferably the genes encoding the cytokine and the antigens are introduced using an expression vector for use in mammalian cells. Such vectors ordinarily include an origin of replication (as necessary, see below), a promoter located in front of the gene to be expressed, optionally an enhancer in trans, along with any necessary ribosome binding sites, RNA splice sites, polyadenylation site, and transcriptional terminator sequences. Such an expression vector may then be used to mediate expression of the at least one antigen and the at least one cytokine.

In a preferred embodiment the expression vector of the present invention comprises, essentially consists or consists of plasmids; phagemids; phages; cosmids; artificial chromosomes, in particular artificial mammalian chromosomes; knock-out or knock-in constructs; viruses, in particular adenovirus, vaccinia virus, attenuated vaccinia virus, canary pox virus, herpes virus, in particular Herpes simplex virus, retrovirus, adeno-associated-virus, rhinovirus, filovirus, and engineered versions of above mentioned viruses; virosomes; "naked" DNA, liposomes; virus-like particles; and nucleic acid coated particles, in particular gold spheres. The retrovirus is, preferably, a lentivirus.

Examples of plasmids, which allow the generation of such recombinant viral vectors include pFastBac1 (Invitrogen Corp., Carlsbad CA), pDCCMV (Wiznerowicz et al., „Double-copy bicistronic retroviral vector platform for gene therapy and tissue engineering: application to melanoma vaccine development." GeneTher. 1997 Oct;4(10):1061-8.) and pShuttle-CMV (Q-biogene, Carlsbad, California). Particularly preferred are viral vectors like lentiviral vectors (LV), retroviral vectors, adenoviral vectors.

LV offer an approach by which efficient, long lasting, non-toxic, and non-immunogenic gene delivery into monocytes and DC may be obtained. Lentiviruses can infect non-proliferating cells, due to the karyophilic properties of the lentiviral pre-integration complex, which allows recognition by the cell nuclear import machinery. LV can transduce primary quiescent cells, cells that are growth-arrested in culture, as well as terminally differentiated cells. The lentiviral packaging system was originally developed by Naldini *et al.* following a tripartite transient transfection procedure Naldini L et al. "In vivo gene delivery and stable transduction of nondividing cells by a lentiviral vector". *Science.* Apr 12 1996;272(5259):263-267) and later evolved into the "second generation" LV, where the four accessory genes of HIV (vif, vpr, vpu and nef) were deleted from the viral packaging system without affecting viral titers or transduction efficiency (Dull T et al. "A third-generation lentivirus vector with a conditional packaging system". *J Virol.* 1998;72(11):8463-8471). The only remaining auxiliary gene in this system is therefore *rev*, which, along with the Rev response element (RRE), as its cognate binding sequence, is required for efficient export of the vector and packaging construct RNAs from the nucleus during virus production. Thus both toxicity as well as the likelihood of recombination are reduced in the "second-generation" LV. In parallel with improvements in the packaging system, has been the development of *self-inactivating (SIN) LV* designs which generally contain a 400 nucleotide deletion in the 3' long terminal repeat (LTR) (Zufferey R et al. "Self-inactivating lentivirus vector for safe and efficient in vivo gene delivery". *J Virol.* Dec 1998;72(12):9873-9880.). The risk of vector mobilization with the wild type virus and subsequent production of replication competent LV is drastically reduced for the SIN vectors. The self-inactivating vectors with a tat-independent promoter have been termed *"third generation" LV.* In most cases, LV are pseudotyped (*i.e.*, encoated with a heterologous envelope protein) with vesicular stomatitis virus glycoprotein (VSV-G), which is a rhabdovirus envelope protein that is reported to bind to ubiquitous cell surface phospholipids, thereby achieving a wide host range.

Thus, in a preferred embodiment of the present invention the lentiviral vector is a first generation LV, a second generation LV or a third generation LV. In an especially preferred embodiment, the LV is a third generation, i.e. self-inactivating, LV as this type of LV has a superior safety profile. The LV is, preferably pseudotyped.

An especially preferred integrase-defective lentiviral vector has a mutated integrase with a nucleic acid sequence as defined by SEQ ID NO: 1.

The use of integrase-defective lentiviral vectors increases the safety of the iDC because integration events may cause insertional mutagenesis with consequences which are difficult to assess. Surprisingly, it has been found in the study underlying the present invention that integrase-defective vectors still allow for a stable and enduring expression of the antigen and the cytokine in differentiated DC even though the vectors may get lost during cell division and are unable to replicate on their own.

In cases where an adenovirus is used as an expression vector, the coding sequences may be ligated to an adenovirus transcription/translation control complex, e.g., the late promoter and tripartite leader sequence. The genes of interest may be inserted in the genome of an adenovirus by *in vitro* or *in vivo* recombination. Insertion in a non-essential region of the viral genome (e. g., region El, E3, or E4) will result in a recombinant virus that is viable and capable of expressing the respective hypercytokine in infected cells. It is preferred that the viral vector used is modified to be replication incompetent.

To allow stable expression of a transgene the expression vector either has to be provided with an origin of replication, which allows replication independent from the genome of the cell, or has to be integrated into the genome of the first and/or second cells or it can be maintained stable as an episome in the nucleus of non-replicating cells. In the first case the expression vector is maintained episomally. Suitable origins of replication may be derived from SV40 or other viral (e. g., Polyoma, Adeno, CMV, VSV, BPV) source. In the second case, if the expression vector is integrated into the genome, e.g. a chromosome, it is not required to provide an origin of replication. In the later case, the episome corresponds to the pre-integration complex containing the double-stranded DNA copy of a retrovirus, lentivirus or more specifically HIV. Thus, it has been surprisingly found in the study underlying the present invention that integrase-defective lentiviral vectors which do neither replicate episomally nor integrate into the genome of the iDC are suitable for inducing self-differentiation of the iDC and maintaining their viability for several weeks and for supporting the regeneration of the immune system in an immunodeficient host following transplantation of HSC. Therefore, replication-incompetent vectors which do not integrate into the genome of the host cell are especially preferred.

To direct expression of the at least one cytokine and the at least one antigen, the genes encoding them are operationally linked to an internal promoter and/or enhancer that is recognized by the transcriptional machinery of the cell. Suitable promoters may be derived from the genome of mammalian cells (e. g., MHCII promoter, EF1alpha promoter) or from mammalian viruses (e.g., the cytomegalovirus promoter, the spleen focus-forming virus SFFV promoter). Especially preferred are promoters which enable the expression of the above-mentioned genes in dendritic cells or their progenitor cells.

In principle, every promoter which is transcriptionally active in antigen presenting cells can be used for the construction of lentiviral vectors which enable expression of antigens and cytokines in iDC or their progenitor cells. Thus, the promoter is, preferably, selected from the group consisting of costimulatory ligands (B7.1/CD80, B7.2/CD86, CD70), DC maturation markers (CD83) and DC markers (CD1c/ BDCA-1, CD141/BDCA-3, CD209, CD40).

Mouse bone marrow progenitors transduced with LV expressing GM-CSF and IL-4 driven by the constitutive early cytomegalovirus (CMV) promoter or by the antigen-presenting cell restricted major histocompatibility complex class II (MHCII) promoter effectively induced DC self-differentiation *in*

vitro and *in vivo* (Pincha et al, 2011) (Pincha M et al. Lentiviral vectors for induction of self-differentiation and conditional ablation of dendritic cells. *Gene Ther. 2011,*Aug;18(8):750-764.). Therefore, these two promoters are especially preferred. Moreover, the use of other commonly used constitutive promoters (SV40, UBC, EF1A, PGK and CAGG) is preferred for the LV design for production of human iDC. Promoters that can be induced, for example, with doxycicline or tamoxifen can also be used.

The early and late promoters of SV40 virus are particularly useful because both are obtained easily from the virus as a fragment which also contains the SV40 viral origin of replication. Smaller or larger SV40 fragments may also be used, provided there is included the approximately 250 bp sequence extending from the *Hind*III site toward the *Bgl*II site located in the viral origin of replication.

As used herein, "operatively linked" means incorporated into a genetic construct so that expression control sequences effectively control expression of a coding sequence of interest.

Specific initiation signals may also be required for efficient translation of coding sequences. These signals include the ATG initiation codon and adjacent sequences. Exogenous translational control signals, including the ATG initiation codon, may additionally need to be provided. One of ordinary skill in the art would readily be capable of determining this and providing the necessary signals. It is well known that the initiation codon must be in-frame (or in-phase) with the reading frame of the desired coding sequence to ensure translation of the entire insert. These exogenous translational control signals and initiation codons can be of a variety of origins, both natural and synthetic.

In order to allow co-expression of several cytokines and antigens in a single multicistronic RNA by mammalian cells, vector designs containing "2A" elements can be employed. The 2A-like sequences are highly conserved naturally occurring viral elements and are short polypeptide sequences (approximately 20 amino acid long) containing a consensus motif (2A, Asp-Val/Ile-Glu- X- Asn- Pro-Gly; 2B, Pro), resulting in cleavage between the 2A glycine and 2B proline. The cleavage is thought to occur by a ribosomal 'skipping' mechanism in which the 2A elements modify the ribosomal activity to skip a peptide bond formation between the glycine and the proline residues, resulting in the release of individual multiple protein products (Donnelly ML et al. "Analysis of the aphthovirus 2A/2B polyprotein 'cleavage' mechanism indicates not a proteolytic reaction, but a novel translational effect: a putative ribosomal 'skip'." *J Gen Virol.* May 2001;82(Pt 5):1013-1025). Recent studies have utilized the 2A-like *cis*-acting hydrolase elements (CHYSEL) to create multicistronic vectors for simultaneous co-expression of multiple genes as individual proteins from a single open reading frame (ORF) transcript (Chinnasamy D et al. "Multicistronic lentiviral vectors containing the FMDV 2A cleavage factor demonstrate robust expression of encoded genes at limiting MOI". *Virol J.* 2006;3:14; Szymczak AL, Vignali DA. "Development of 2A peptide-based strategies in the design of multicistronic vectors". *Expert Opin Biol Ther.* May 2005;5(5):627-638). A

particular advantage of the 2A system in construction of multicistronic vectors is the feasibility of using only a single promoter. Due to their small size, a single vector construct can utilize several 2A elements for expression of multiple proteins. 2A-like elements allow expression of multiple protein products at high efficiency of cleavage at equimolar ratios (de Felipe P. et al. "E unum pluribus: multiple proteins from a self-processing polyprotein". *Trends Biotechnol.* Feb 2006;24(2):68-75). Heterologous 2A elements in the vector avoid homologous recombination, maintaining stability of the lentiviral vector. Different types of 2A-elements that can be used in the vector include: foot-and-mouth disease virus (F2A), equine rhinitis A virus (E2A), *Thosea asigna* virus (T2A) and porcine teschovirus-1 (P2A).

Alternatively, internal ribosome entry sites (IRES) can be used. Usually, in eukaryotes, translation can be initiated only at the 5' end of the mRNA molecule, since 5'-cap recognition is required for the assembly of the translation initiation complex. An IRES, is a nucleotide sequence that allows for translation initiation in the middle of a messenger RNA (mRNA) sequence by a process called internal translation initiation. Viral IRES are found for example in Picornavirus, hepatitis virus or poliovirus. Cellular IRES are found for example in the mRNAs encoding for fibroblast growth factor (FGF), platelet derived growth factor (PDGF), vascular endothelial growth factor (VEGF), insulin-like growth factor 2 (IGF-II), C-myc, L-myc, Pim-1, protein kinase, p58PITSLRE or p53. Similar to 2A elements, heterologous IRES can be combined in a vector for translation of one mRNA into several protein products.

Thus, in a vector encoding more than one polypeptide, e.g. an antigen and a cytokine, the nucleic acid sequence encoding the polypeptides are, preferably separated by a 2A element or an IRES. 2A elements which are especially preferred for use in the present invention are defined by SEQ ID NOs: 5 and 6.

The efficiency of expression may be enhanced by the inclusion of appropriate transcription enhancer elements and transcription terminators. In eukaryotic expression, one will also typically desire to incorporate into the transcriptional unit an appropriate polyadenylation site (e.g., 5'-AATAAA-3') if one was not contained within the original cloned segment. Typically, the poly A addition site is placed about 30 to 2000 nucleotides "downstream" of the termination site of the protein at a position prior to transcription termination.

Antigens

Preferably, the antigen expressed by the iDC is an antigen that can induce a cytotoxic or humoral immune response selected from the group consisting of xeno-reactivity, allo-reactivity, neo-reactivity or auto-immunity.

The term "xeno-reactivity" refers to the eliciting of an immune response against a protein expressed by an organism from another species, preferably a pathogen.

The term "allo-reactivity" refers to the eliciting of an immune response against a protein expressed by cells or tissues transplanted from a donor from the same species.

The term "neo-reactivity" refers to the eliciting of an immune response against a mutated protein of the subject receiving the iDCs. Preferably, neo-reactivity is directed at a cancer-antigen.

The term "auto-immunity" refers to the eliciting of an immune response against a protein expressed in the body of the organism from which the immune cells mediating said immune response are derived. Typically auto-immunity is caused by the preclusion of mechanisms of immune tolerance. Preferably, auto-immunity is directed at a protein which is abnormally over-expressed by cancer cells without being mutated.

Especially preferred antigens inducing xeno-reactivity are selected from the group consisting of pp65 (derived from human cytomegalovirus), NS3 (derived from hepatitis C virus) and gag and env (derived from human immunodeficiency virus).

Especially preferred cancer-antigens, i.e. antigens eliciting neo-reactivity or auto-immunity, are selected from the group consisting of TRP2, MART1, WT1 and Tyrosinase (all derived from melanoma) and WT1, Her2/neu and BRCA1/2 (all derived from breast cancer).

Especially preferred is the use of pp65 as encoded by the nucleic acid represented by SEQ ID NO: 2. It is to be understood that the genetic code is degenerate so that different triplets encode the same amino. Hence, the invention also relates to all nucleic acid sequences encoding the same amino acid sequence as SEQ ID NO: 2. Since different triplets encoding the same amino acid are translated with different efficiency in different species, the optimization of the nucleic acid sequence by codon optimization is envisaged by the present application.

Moreover, at least one antigen encoded by the nucleic acid sequence comprised by the vector may be a variant of one of the aforementioned antigens which is immunologically identical to the antigen

The term "variant" with respect to an antigen refers to proteins derived by deletion and/or substitution of at least 1, 2, 3, 4, 5, 8, 10, 15 or 20 amino acids from the antigen. Preferably, the variant hast at least 80 %, 85 %, 90 %, 95 %, 96 %, 97 %, 98 %, 99 %, 99.5 % 99.7 %, 99.8 % or 99.9 % sequence identity to the antigen. Especially preferred are variants which are derived by C-terminal and/or N-terminal deletion of at least 1, 2, 3, 4, 5, 8, 10, 15 or 20 amino acids.

Moreover, the term "variant" refers to fragments of the above-described antigens which retain at least 5, 10, 15, 20, 25, 30, 40, 50, 60, 70, 80 or 90 amino acids of the full-length antigen.

It is preferred that all variants or fragments of the antigen are immunologically identical to the antigen. Two or more antigens are "immunologically identical" if they are recognized by the same antibody, T-cell or B-cell. The recognition of two or more immunogenic polypeptides by the same antibody, T-cell or B-cell is also known as "cross reactivity" of said antibody, T-cell or B-cell. Preferably, the recognition of two or more immunologically identical polypeptides by the same antibody, T-cell or B-cell is due to the presence of identical or similar epitopes in all polypeptides. Similar epitopes share enough structural and/or charge characteristics to be bound by the Fab region of the same antibody or B-cell receptor or by the V region of the same T-cell receptor. The binding characteristics of an antibody, T-cell receptor or B-cell receptor are, preferably, defined by the binding affinity of the receptor to the epitope in question. Two immunogenic polypeptides are "immunologically identical" as understood by the present application if the affinity constant of polypeptide with the lower affinity constant is at least 30 %, at least 40 %, at least 50 %, at least 60 %, at least 70 %, at least 80 %, at least 90 %, at least 95 % or at least 98 % of the affinity constant of the polypeptide with the higher affinity constant. Methods for determining the binding affinity of a polypeptide to a receptor such as equilibrium dialysis or enzyme linked immunosorbent assay (ELISA) are well known in the art. Preferably, two or more "immunologicaly identical" polypeptides comprise at least on identical epitope.

Cytokines

The cytokine expressed by the iDC is any cytokine which is – on its own or in combination with one or more additional cytokines – able to induce the differentiation of progenitor cells into dendritic cells. Moreover, it is required that an iDC expressing the at least one cytokine and the at least one antigen is suitable for the methods and uses presented further below in the present application. Particularly, the DC must express all cytokines which support the regeneration of a functional immune system in an immunodeficient host after stem cell transplantation.

Preferably, the iDC expresses at least one cytokine selected from the group consisting of GM-CSF, IL-4, IFN- α, IL-15, TGF-B, TNF-α, FLT3L, IL-3 and CD40L.

More preferably, the iDC expresses a combination of cytokines selected from the group of combinations consisting of (i) FLT3L and IL-3; (ii) FLT3L and CD40L; (iii) FLT3L and IFN α; (iv) GM-CSF and IFN-α and IL-15; (v) GM-CSF and IFN-α and TNF-α; and (vi) GM-CSF and IFN-α and TGF-B.

Most preferably, the iDC expresses a combination of cytokines selected from the group of combinations consisting of (i) GM-CSF and IFN-α; (ii) GM-CSF and IL-4; and (iii) GM-CSF and IL-15.

A nucleic acid sequence GM-CSF is given in SEQ ID NO: 3 and a nucleic acid encoding IFNα is given in SEQ ID NO: 4. It is to be understood that the genetic code is degenerate so that different triplets encode the same amino. Hence, the invention also relates to all nucleic acid sequences encoding the same amino acid sequence as the amino acid sequences encoded by SEQ ID NOs: 3 and 4. Since different triplets encoding the same amino acid are translated with different efficiency in different species, the optimization of the nucleic acid sequence by codon optimization is envisaged by the present application.

Generation of iDCs

Moreover, the present invention relates to a method for generating iDCs comprising the steps of

a) isolating progenitor cells from a sample derived from a suitable donor;

b) engineering the cells to achieve expression of at least one cytokine which induces the self-differentiation of human DC progenitor cells into DCs and at least one antigen.

Preferably, the above-described method comprises the steps of

a) isolating progenitor cells from a sample derived from a suitable donor;

b) incubating the progenitor cells in a suitable medium in the presence of cytokines which enhance a gene transfer method and/or stimulate the development of the progenitor cells into dendritic cells.

c) transfecting or transducing the cells with at least one vector which is suitable for the expression of at least one cytokine which induces the self-differentiation of human DC progenitor cells into DCs and at least one antigen.

All the definitions given above, also apply to this embodiment.

A cytokine which enhances a gene transfer method is a cytokine which – when added to the growth medium before or during transfection – increases the efficiency of the uptake the foreign nucleic acid. Preferred for this purpose are GM-CSF, IL-4 or a combination thereof.

The iDCs generated by this method may be cultured until further use in a suitable medium. They may, however, also be stored frozen until they are administered to the patient. Methods for the cryopreservation of viable cells are known to the person skilled in the art.

In a preferred embodiment of the present invention, the progenitor cells are peripheral blood monocytes expressing CD14 derived from a human donor and the medium in method step a) is supplemented with GM-CSF and IL-4 or with GM-CSF and IFNα. This embodiment, preferably, involves use of integrase-defective lentiviral vectors for the expression of the aforementioned cytokines and at least one antigen are used in step c). Preferably, the antigen is pp65. This embodiment is described in greater detail in the examples section.

Therapeutic uses of the iDCs of the present invention

Furthermore, the present invention relates to an iDC as described above for use as a medicament.

Moreover, the invention relates to an iDC as described above for use in the regeneration of the immune system of an immunodeficient subject following transplantation of hematopoietic stem cells (HSC).

Preferably, the subject is an organism selected from the group consisting of primate, rodent, cat, dog, pig, cow and sheep. The primate is, preferably, a human, a chimpanzee or a macaque, most preferably a human. The rodent is, preferably, a mouse or rat, most preferably a mouse.

The mouse belongs, preferably, to a strain selected from the group consisting of NOD-Rag1^{null}IL2Ry^{null}-NRG, NOD/LtSz-SCID/IL2Ry^{null}-NSG and NOD/SCID/IL2Ry^{null}-NOG. Older mouse strains that were used for generation of humanized mouse such as Balb/c-Rag1^{null}IL2Ry^{nul} ; NOD-SCID, NOD-SCID β2m^{null} are also preferred. In addition, newer transgenic or knock-in mouse strains expressing human MHCI or MHCII or human cytokines critical for development of human cells in mice (such as GM-CSF, IL-3, IL-7, IL-15) could also be used.

Immunodeficiency

The term "immunodeficiency" refers to any condition of the subject's immune system which is characterized by an impaired immune response as compared to a average individual of the same species which matches the subject with respect to age, sex and general living conditions such nutritional state. Said immunodeficiency is, preferably, caused by decreased numbers or an impaired function of at least one of the groups of immune cells defined below resulting in an increased susceptibility of the subject to infectious diseases caused by bacteria, viruses, fungi or other unicellular eukaryotes or increased susceptibility to malignancies.

The immune system comprises two major branches, the adaptive immune system and the innate immune system.

The responses of the innate immune system against pathogens are not specific for a given pathogen. The innate immune system rather relies on pattern recognition receptors specific for molecular structures common to a large number of pathogens. Cells which mediate innate immune responses are mast cells, phagocytes (dendritic cells, macrophages and neutrophils) and natural killer cells.

The responses of the adaptive immune system are specific for a given antigen and, therefore, also restricted to this antigen. The cells of the adaptive immune system have antigen-receptors which are formed individually in each cell during its maturation in a random fashion. Thus, the adaptive immune system comprises a large pool of cells with many different receptors. If the subject encounters an antigen proliferation of those subpopulations of cells which carry a receptor specific for an antigen of the pathogen is induced. Later on, once the pathogen is eliminated from the body some of these cells form a population of memory cells, which allow for a more rapid build-up of an immune response if the pathogen is encountered again.

The adaptive immune response is mediated by T-lymphocytes and B-lymphocytes.

There are two major groups of T-lymphocytes: (i) T-helper cells, characterized by expression of the CD4-receptor on their surface, produce a variety of cytokines once their receptor binds a suitable antigen. This cytokines activate other cells of the immune system. Particularly, the microbicidal function of macrophages and the secretion of antibodies by B-lymphocytes is stimulated. (ii) Cytotoxic T cells (CTL), characterized by expression of the CD8-receptor on their surface, recognize and kill cells which are infected by viruses or other pathogens and recognize and kill tumor cells. CTL activation against an antigen presented by the major histocompatibility complex I (MHCI) is facilitated by T helper cells

B-lymphocytes secrete antibodies which are specific for the antigen which is recognized by their receptor.

Depending on its cause, the immunodeficiency may be permanent or transient. A primary immunodeficiency results from inborn genetic aberrations is typically permanent. If the immunodeficiency is caused by ionizing radiation or a cytotoxic pharmaceutical, its duration depends on the dosage of the pharmaceutical or the radiation. If the dosage is sufficiently high to kill all hematopoietic stem cells in the subject, the immunodeficiency will be permanent. If only a part of the hematopoietic stem cells is killed, the immunodeficiency will resolve, once the remaining hematopoietic stem cells are able to repopulate the different hematopoietic lineages.

Preferably, the immunodeficiency of the subject is caused by is an immunodeficiency selected from the group consisting of immunodeficiency caused by ionizing radiation, immunodeficiency caused by the administration of at least one cytotoxic pharmaceutical, primary immunodeficiency and immunodeficiency caused by a pathogen. It is also envisaged that two or more of the aforementioned immunodeficiencies may be present in the subject.

An immunodeficiency caused by ionizing radiation may be caused by accidental or deliberate exposure to said radiation. Typically, deliberate exposure to ionizing radiation takes place as a treatment of cancer. Ionizing radiation predominantly kills those cells which are rapidly dividing. Thus, it is suited for the therapy of a variety of cancers as cancer cells are in many cases characterized by rapid proliferation. However, the cells of the immune system including the hematopoietic stem cells are also highly sensitive to ionizing radiation. Thus, they may be damaged in the course of radiotherapy of cancer. This damage may be an undesired side effect of organ- or tissue-specific irradiation of a solid cancer. However, especially in the case of leukemia which arises from the degeneration of stem cells of the immune system, a complete eradication of all hematopoietic stem cells is often the last option. In this case, transplantation of allogeneic hematopoietic stem cells after irradiation is required for the survival of the patient.

Typical types of ionizing radiation employed in the radiation therapy of cancer are X-rays, electron beams (energy range 4 to 20 MeV), particle radiation (protons, neutrons, ionized nuclei) and gamma radiation originating from sources such as cobalt-60, radium-226, caesium-137 or iridium-192.

A primary immunodeficiency is caused by genetic defect which impairs or completely inhibits the development of at least one type of immune cells in the subject.

Preferably, the primary immunodeficiency is selected from the group consisting of X-linked severe combined immunodeficiency (SCID), adenosine deaminase deficiency and IL-7Rα–chain deficiency. Preferably, X-linked SCID is caused by a mutation of IL-2RG.

It is to be understood that the immunodeficiency treated by the transplantation of hematopoietic stem cells may also be a primary immunodeficiency which is aggravated by ionizing radiation and/or the administration of at least one cytotoxic pharmaceutical.

Immunodeficiency caused by the administration of at least one cytotoxic pharmaceutical is also a typical effect of cancer therapy. Cytotoxic pharmaceuticals employed in cancer therapy frequently kill immune cells (including hematopoietic stem cells) in addition to cancer cells. With the dosages of cytotoxic pharmaceuticals usually employed, the immunodeficiency is, typically, transient as not all hematopietic stem cells in the patient are killed. Nevertheless, if hematopoietic stem cell

transplantation is envisaged as a cure for this condition, the method of the present invention will be helpful.

In severe cases of certain cancers (e.g. Hodgkin`s lymphoma) which do not respond to other types of treatment anymore a type of chemotherapy termed "high dosage chemotherapy" is employed. While the dosages of cytotoxic pharmaceuticals in other chemotherapy regimens are selected in order to minimize the resulting immunodeficiency, the complete destruction of the subject's immune system is a planned effect of high dosage chemotherapy. For this reason the subject receives an autologous or allogeneic hematopoietic stem cell transplant after completion of high dosage chemotherapy.

Typical cytotoxic pharmaceuticals include any inhibitors of topoisomerase I or II, such as camptothecin (topo I) or etoposide (topo II); any compound that interchelates DNA, such as doxorubicin. Particularly preferred are alkylating substances, anti-metabolites, antibiotics, epothilones, nuclear receptor agonists and antagonists, platinum compounds, interferons and inhibitors of cell cycle-dependent protein kinases (CDKs), inhibitors of cyclooxygenases and/or lipoxygenases, platinum coordination complexes, ethyleneimenes, methylmelamines, trazines, vinca alkaloids, pyrimidine analogs, purine analogs, alkylsulfonates, folic acid analogs, anthracendiones, substituted urea, methylhydrazin derivatives, in particular acediasulfone, aclarubicine, ambazone, aminoglutethimide, L-asparaginase, azathioprine, bleomycin, busulfan, calcium folinate, carboplatin, carpecitabine, carmustine, celecoxib, chlorambucil, cis-platin, cladribine, cyclophosphamide, cytarabine, dacarbazine, dactinomycin dapsone, daunorubicin, dibrompropamidine, diethylstilbestrole, docetaxel, doxorubicin, enediynes, epirubicin, epothilone B, epothilone D, estramucin phosphate, etoposide, flavopiridol, floxuridine, fludarabine, fluorouracil, fluoxymesterone, flutamide fosfestrol, furazolidone, gemcitabine, hexamethylmelamine, hydroxycarbamide, hydroxymethylnitrofurantoin, hydroxyprogesteronecaproat, hydroxyurea, idarubicin, idoxuridine, ifosfamide, irinotecan, leuprolide, lomustine, lurtotecan, mafenide sulfate olamide, mechlorethamine, megastrolacetate, melphalan, mepacrine, mercaptopurine, methotrexate, metronidazole, mitomycin C, mitopodozide, mitotane, mitoxantrone, mithramycin, nalidixic acid, nifuratel, nifuroxazide, nifuralazine, nifurtimox, nimustine, ninorazole, nitrofurantoin, nitrogen mustards, oleomucin, oxolinic acid, pentamidine, pentostatin, phenazopyridine, phthalylsulfathiazole, pipobroman, prednimustine, preussin, procarbazine, pyrimethamine, raltitrexed, rapamycin, rofecoxib, rosiglitazone, salazosulfapyridine, scriflavinium chloride, semustine streptozocine, sulfacarbamide, sulfacetamide, sulfachlopyridazine, sulfadiazine, sulfadicramide, sulfadimethoxine, sulfaethidole, sulfafurazole, sulfaguanidine, sulfaguanole, sulfamethizole, sulfamethoxazole, co-trimoxazole, sulfamethoxydiazine, sulfamethoxypyridazine, sulfamoxole, sulfanilamide, sulfaperin, sulfaphenazole, sulfathiazole, sulfisomidine, staurosporin, tamoxifen, taxol, teniposide, tertiposide, thioguanine, thiotepa, tinidazole, topotecan, triaziquone, treosulfan, trimethoprim, trofosfamide, UCN-01, vinblastine, vincristine, vindesine, vinblastine, vinorelbine, and zorubicin, or their respective derivatives or analogues thereof.

Immunodeficiency caused by pathogen is caused by infection of the subject with a virus or a bacterium. Said pathogen causes an immunodeficiency if it interacts directly with cell of the immune system, e.g. by using them as host cells, or by indirect means. Indirect means include the secretion of substances which modulate the activity of immune cells or the generation of mature immune cells from their progenitor cells. Moreover, a pathogen may induce secretion of such compounds by cells of the host organism. Immunodeficiency caused by a pathogen as referred to in this application is preferably caused by an infection with the human immunodeficiency virus (HIV)

Regeneration of the immune system

The term "regenerating the immune system in an immunodeficient subject" relates to the improvement of the overall function of the immune system of an immunodeficient subject.

Preferably, the number and/or activity of at least one type of immune cells are increased. More, preferably, the activity of the adaptive or the innate immune system is increased. Also preferably, the activity and/or number of B-lymphocytes or T-lymphocytes are increased.

Even more preferably, the number of immune cells in and/or the activity of both branches of the immune system are increased.

Most preferably, as a result of the regeneration of the immune system, there are no significant differences between the function of the immune system of a healthy individual matched for age, sex and nutritional state and the subject whose immune system has been regenerated by transplantation of HSC and administration of the iDC of the present invention. Thus, most preferably, the regeneration of the immune system fully cures the immunodeficiency.

However, it is envisaged that the recipient of the transplanted HSC may be in need of immunosuppressive therapy in order to suppress a rejection of the transplanted HSC by the remainder of the host's immune system or to counter graft-versus-host disease. The necessity of such immunosuppressive therapy does not render the regeneration of the immune system less complete as this term as understood in this application only refers to the potential function of the immune system resulting from the transplanted HSC.

Transplantation of HSC

The term "transplanting hematopoietic stem cells" refers to the process of deriving material comprising hematopoietic stem cells (HSC) from one subject ("donor") and administering a preparation comprising HSC to the same or a different subject ("recipient").

In the simplest case, the material comprising HSC produced from the donor is administered to the recipient without further treatment steps. HSC preparations derived from umbilical cord blood and bone marrow may be administered to the subject directly without enrichment or depletion of cell types comprised therein. In these cases, the preparation comprising HSC is identical to the material taken from the donor.

However, it is preferred to produce the preparation administered to the recipient by depleting T-lymphocytes from the material derived from the donor. This can be achieved by removing cells which are CD8-positive and/or CD3-positive from the material produced from the donor. It is also preferred that cells which are CD34-positive are enriched the preparation of HSC administered to the subject. If the HSC are derived from peripheral blood, it is preferred to enrich cells which are CD34-positive prior to administration to the subject. Means and methods for depletion or enrichment of cells carrying specific surface antigens are conventional in the art and, e.g. described in the examples section.

The HSC transplanted to the subject may be produced by every method known in the art. Typically, bone marrow of the donor has been the source of HSC for transplantation. In this method, the HSC are removed from a large bone of the donor.

However, production of stem cells from peripheral by apheresis has become the most common method. Before the HSC are produced, the donor receives granulocyte colony stimulating factor in order to increase the amount of HSC in peripheral blood. Apheresis is used to separate the white blood cells (including the HSC) from the whole blood. The white blood cells are stored for use in transplantation while plasma and erythrocytes returned to the donor's circulation.

A further source of HSC is umbilical cord blood. Umbilical cord blood is produced from the placenta and the umbilical cord after birth. Umbilical cord blood has a higher concentration of HSC than normally found in adult peripheral blood.

In the context of the present invention it is preferred that the stem cells are derived from one of the three aforementioned sources.

The transplantation of HSC is autologous or allogeneic.

In autologous stem cell transplantation donor and recipient of the HSC are identical, i.e. the HSC comprised by the preparation administered to the subject are derived from the same subject. Autologous transplantation of HSC is preferred if the immunodeficiency of the subject is caused by high dosage chemotherapy. In this case, material comprising HSC can be produced from the subject before the onset of high dosage chemotherapy and the preparation comprising HSC can be administered to the subject after completion of high dosage chemotherapy.

Allogeneic transplantation of HSC is involves the production of material comprising HSC from one subject and the administration of a preparation comprising HSC of the first subject to a different subject. Thus, donor and recipient are not identical. A special case of allogeneic transplantation of HSC is xenogeneic transplantation of HSC. In this case, donor and recipient of the HSC belong to different species.

Heterologous transplantation of HSC is, typically, performed if the subject suffers from leukemia, myeloma, severe combined immunodeficiency, aplastic anemia or congenital neutropenia. In these cases, a donor is selected who shares as many immunological properties with the recipient as possible. Especially important are identical HLA-A, HLA-B, HLA-C, HLA-DR and HLA-DQB1 genes. Thus, in one preferred embodiment of the present invention, the transplantation of HSC is allogeneic transplantation, wherein the donor and recipient are human.

In another preferred embodiment, the transplantation of HSC is xenogeneic, wherein the donor is a human and the recipient is a rodent, preferably a mouse having a primary immunodeficiency, most preferably severe combined immune deficiencies SCID such as X-linked SCID (with mutations in *IL-2RG*) or in enzymes derived from the recombination activating genes (RAG-1 and RAG-2).

Moreover, the present invent invention relates to an iDC as described above for use as a medicament for the treatment of cancer which spreads lymphatically or a disease caused by a lymphotrophic pathogen.

The iDC of the invention migrate preferentially through the lymphatic vessels to the lymph nodes. For this reason, they are especially suited for the treatment of diseases which involve the lymph nodes.

One hallmark of cancer is its ability to spread systemically throughout the body resulting in the formation of metastases. Once metastases are present, surgical resection or irradiation of the primary tumor are not sufficient to cure the disease. If there are few metastases, surgery and/or radiotherapy may be used to remove said metastases. However, in most cases, the number of metastases is too disseminated to allow for a removal by the aforementioned means. At that point of a cancer disease, systemic therapy such as chemotherapy is the only means of treatment left which maintains the chance of a cure. However, in many cases chemotherapy fails to kill all cancer cells or the cancer acquires resistance against the drug. Therefore, it is of pivotal importance to prevent the systemic spread of cancer cells.

Two major routes exist for the systemic spread of cancer: the circulatory system and the lymphatic system. In the latter case cells of the tumor migrate into the lymphatic vessels draining the area, where the tumor is located. The cells than migrate to the regional lymph nodes. These lymph nodes are also

known as "sentinel lymph nodes". The analysis of lymph nodes draining a specific area is routine part of the surgical resection of a tumor and the presence or absence of cancer cells in the sentinel lymph nodes is an important parameter for the staging of the disease and the selection of the appropriate treatment. The presence of cancer cells in the sentinel lymph nodes is associated with a bad prognosis.

The iDC of the present invention have the potential to mediate the killing of cancer cells which are present in the lymph nodes, thus blocking further lymphatic spread of the disease. For this use of the iDC it is preferred that they express an antigen presented by the cancer cells in question.

Preferred cancers that are treated according to the invention are selected from the group consisting of melanoma and breast cancer, hematologic malignancies (leukemia, lymphoma), neurologic malignancies (glioma, glioblastoma), prostate cancer, lung cancer, colon cancer and liver cancer. The treatment of melanoma and breast cancer is especially preferred.

Preferably, an iDC for the treatment of cancer which spreads lymphatically expresses at least one antigen which is selected from the group consisting of TRP2, MART1, WT1 and Tyrosinase (all derived from melanoma) and WT1, Her2/neu and BRCA1/2 (all derived from breast cancer).

There are pathogens which are primarily or even exclusively found in the lymphatic system. For the use in the therapy of such a disease, the iDC, preferably, expresses at least one antigen also expressed by the respective pathogen.

The pathogen is, preferably, selected from the group consisting of viruses, bacteria, fungi and unicellular eukaryotes. More preferably, it is a virus, most preferably HIV. Preferably, iDC for use in the treatment of HIV express the antigens gag and env.

Method for regenerating an immune system

Furthermore, the present invention relates to a method for regenerating an immune system in an immunodeficient subject comprising the steps of

a) transplanting hematopoietic stem cells to the subject; and

b) administering to the subject an iDC engineered to express at least one antigen and at least one cytokine which induces the self-differentiation of human DC progenitor cells into DCs.

All definitions given above for the iDCs and their therapeutic uses also apply to this embodiment.

The above-described method is, preferably, used to generate mice with a humanized immune system. Therefore, the subject is, preferably, a mouse. More preferably, the mouse belongs to a strain selected from the group consisting of NOD-Rag1^{null}IL2Ry^{null}-NRG, NOD/LtSz-SCID/IL2Ry^{null}-NSG and NOD/SCID/IL2Ry^{null}-NOG.

Preferably, the HSC are derived from a human and the antigen is pp65. Moreover, it is preferred that the iDC express the antigen pp65 and the cytokines (i) GM-CSF and (ii) interferon-α or interleukin-4 or interleukin-15. More preferably, this expression is mediated by a vector and, most preferably, it is mediated by an integrase-defective lentiviral vector.

The mice generated by this embodiment have humanized immune system which has an improved function as compared to mice that did not receive the iDC of the present invention before or after transplantation of HSC.

This type of xenogeneic HSC transplantation creates a human immune system in a rodent, preferably a mouse. Such an animal with a human immune system is a valuable tool for research which can be used for certain experiments which are – for ethical or logistic reasons – not possible in humans. Unfortunately, this animal model has some deficiencies. After transplantation of human HSC lymphatic tissue develops only poorly. Surprisingly, administration of human iDC of the present invention to mice after transplantation of human HSC lead to improved reconstitution of lymph nodes containing human T cells and DC in the treated mice, thus making these mice a better model of the human immune system.

Currently available mouse models based on the humanized immune systems (HIS) show poor development of functional T cells, particularly functional cytotoxic T cells, capable of recognizing and lysing target cells presenting antigenic epitopes through the major histocompatibility complex (MHC). In contrast to this, mice with a human immune system generated according to the present invention show an increase in the frequency and an improved T-lymphocyte activity.

Therefore, another preferred embodiment of the present invention relates to a mouse with a humanized immune system generated by the method described above and the use of this mouse for the study of the human immune system or for the testing of drugs, implants or devices for their use in humans. A preferred use is the study of the human adaptive immune system with this mouse model

Since it is not desirable that implants cause inflammation in the human body, the testing of implants in the humanized mouse model of the present invention is an especially preferred use.

Brief description of the Figures

Figure 1: **Smyle/pp65 generation and characterization.** (a) CD14+ monocytes from GCSF-mobilized healthy donors were isolated by magnetic selection and co-transduced with LV-GM-IFNα and LV-pp65 for Smyle/pp65 or LV-pp65 alone for conventional DC (Con-IFN/pp65) generation. Con-IFN/pp65 DC were maintained throughout the culture in the presence of recombinant cytokines, i.e., GM-CSF and IFN-α (b) Cell viability represented by averaged percentage of recovery was assessed in cultured Smyle/pp65 or Con-IFN/pp65 at different time points (7, 14 and 21 days). (c) Expression of CMV-pp65 and (d) Stability of dendritic cell phenotype was analyzed by flow cytometry in Smyle/pp65 and Con-IFN/pp65 cultured for up to three weeks. Data represent the average of at least three different donors for every time point. *$p<0.05$

Figure 2: **Cytokines accumulated in Smyle/pp65 (not supplemented with recombinant cytokines) and Con-IFN/pp65 (supplemented with recombinant GM-CSF and IFN-α) cell culture supernatants.** (a) Cytokine pattern after 7 days. (b) Cytokine pattern after 14 days. (c) Cytokine pattern after 21 days

Figure 3: **Smyle/pp65 immunization significantly enhances the frequency of human CD3+ T cells in peripheral blood of HIS-NRG mice.** (a) HIS-NRG mice were generated by transfer of human $CD34^+$ hematopoietic stem cells (HSC). Mice were subcutaneously injected with Smyle/pp65 or Con/pp65 in the right flanks at week 10 and 11 after HSC-reconstitution. (b) Frequency of CD45+/CD3+ human T cells in peripheral blood of control, Smyle/pp65 or Con-IFN/pp65-immunized HIS-mice in weeks 10, 13 and 20 after HSCT.

Figure 4: **Smyle/pp65 immunization significantly enhances the frequency of human CD3+ T cells and effector memory cytotoxic T cells in HIS-NRG mice** (a) Frequency of human $CD45^+$ and $CD3^+$ cells in mononuclear cells obtained from spleen of HIS-NRG mice. (b) Relative frequency of human $CD4^+$ and $CD8^+$ lymphocyte subsets and the corresponding frequencies of $CD45RA^+/CD62L^+$ naïve and effector memory

$CD45RA^-CD62L^-$ subsets in spleens from HIS-mice twenty weeks after HSCT. *$p<0.05$

Figure 5: **Macroscopic detection of lymph nodes after immunization of HIS-NRG with Smyle/pp65.** (a) Macroscopic detection of peripheral lymph nodes (LN) in mice immunized with Smyle/pp65 cells. (b) Frequency of mice showing detectable LN in different parts of the body.

Figure 6: **Immunofluorescence analyses of LN obtained from wild type C57BL/6 and Smyle/pp65 immunized HIS-mice**. Human T cells and DC fill up the LN Anlage in HIS-NRG mice immunized mice.

Figure 7: **Optical imaging analyses for monitoring the migration of SmyleDC/pp65-flUC administered s.c. into HIS-NRG mice previously immunized with SmyleDC/pp65.** (a) Scheme of experiment. (b) Detection of bioluminescence signal in HIS-NRG mice. (c) Quantified bioluminescence signal detected on the same side versus contra-lateral side, where SmyledC/pp65-fLUC was injected.

Figure 8: **Characterization of CMV-specific cytotoxic T cell responses after HIS-NRG immunization** (a) Frequency of human $CD45^+$, $CD3^+$ and $CD19^+$ cells in LN recovered from HIS-NRG immunized with Smyle/pp65. (b) Relative frequencies of human $CD4^+$ and $CD8^+$ T cells $CD45RA^+/CD62L^+$ naïve, effector memory (EM) $CD45RA^-CD62L^-$, central memory (CM) $CD45RA^-CD62L^+$ and $CD45RA^+/CD62L^-$ terminal effector (TE). (c) Cells were obtained from LN isolated from Smyle/pp65-immunized mice and co-cultured for 7 days with autologous Smyle/pp65 or Smyle (without antigen). Re-stimulation was performed by overnight incubation with CMV-pp65 overlapping peptides and IFNγ spots were counted. (d) Human $CD3^+$ cells sorted from splenocytes obtained from control, Con-IFN/pp65 and Smyle/pp65-immunized

HIS-NRG, activated for 72h with human anti-CD2, CD3 and CD28 antibodies in the presence of hIL-7 and hIL-15.

Figure 9: **B cell responses in HIS-NRG mice.** Frequency of human B cells in peripheral blood (a) and spleen (B) and detection of human immunoglobulin G and M in the sera of HIS-NRG.

The following example is merely intended to illustrate the invention. They shall not limit the scope of the claims in any way.

Examples

Materials and Methods

Lentiviral vector construction and integrase-defective lentivirus production

The self-inactivating (SIN) lentiviral backbone vector and the monocistronic vectors expressing the CMV-pp65 and firefly luciferase, LV-fLUC were previously described (Salguero, G. et al., 2011, "Preconditioning therapy with lentiviral vector-programmed dendritic cells accelerates the homeostatic expansion of antigen-reactive human T cells in NOD.Rag1-/-.IL-2rgammac-/- mice." *Hum Gene Ther* 22: 1209-1224). Construction of the bicistronic lentiviral vector expressing the human granulocyte-macrophage colony stimulating factor and of the human interferon alpha (LV-G2α) interspaced with a P2A element (RRL-cPPT-CMV-hGMCSF-P2A-hIL4) was constructed and extensively characterized as previously described (Daenthanasanmak, A. et al., 2012, "Integrase-defective lentiviral vectors encoding cytokines induce differentiation of human dendritic cells and stimulate multivalent immune responses in vitro and in vivo." *Vaccine* 30: 5118-5131). The structural integrity of all constructs was reconfirmed by restriction digestion and sequencing analysis of the promoters and transgenes. Large scale lentivirus production was performed by transient co-transfection of human embryonic kidney 293T cells as formerly described (Stripecke, R., 2009, "Lentiviral vector-mediated genetic programming of mouse and human dendritic cells." *Methods Mol Biol* 506: 139-158.). To generate integrase-defective lentivirus, four packaging plasmids were used in the co-transfection: the plasmid containing the lentiviral vector expressing the cytokines, the plasmid expressing gag/pol containing a D64V point mutation in the integrase gene (pcDNA3g/pD64V.4xCTE), the plasmid expressing rev (pRSV-REV) and the plasmid encoding the VSV-G envelope (pMD.G). Virus supernatants were collected and concentrated by ultracentrifugation and the titers were evaluated by assessing p24 antigen concentration with enzyme-linked immunoabsorbent assay (ELISA) (Cell Biolabs, Inc. San Diego, USA). One µg of p24 equivalent/ ml corresponds to approximately $1x10^7$ infective viral particles/ ml.

Human CD34 positive peripheral blood stem cell isolation

Peripheral blood mononuclear cells (PBMCs) were obtained from leukapheresis of hematopoietic adult stem cell transplantation adult donors subjected to haematopoietic stem cell mobilization regimen with G-CSF (Granocyte, Chugai Pharma). All studies were performed in accordance with protocols approved by the Hannover Medical School Ethics Review Board. CD34+ cells were positively selected by MACS using a CD34 magnetic cell isolation kit (Miltenyi Biotech, Bergisch-

Gladbach, Germany). After two rounds of positive magnetic selection, cell purity obtained was above 97% with a contamination of CD3+ T cells bellow 0.2% as evaluated by flow cytometry.

Generation of human conventional-IFNα and Smyle DCs,

The autologous CD34 negative PBMC fraction was used for further positive selection of $CD14^+$ monocytes using CD14 isolation beads (Miltenyi Biotech). For lentiviral gene transfer, monocytes were kept in culture with serum-free Cellgro medium in the presence of recombinant human GM-CSF and IL-4 (50 ng/ml each, Cellgenix, Freiburg, Germany) for 8 h prior to transduction. For generation of SmyleDC/pp65, $5x10^6$ $CD14^+$ monocytes were transduced at a multiplicity of infection (M.O.I.) of 5 (corresponding to 2.5 µg/ mL p24 equivalent) of both ID-LV-G2α and in the presence of 5 µg/ml protamine sulfate (Valeant, Dusseldorf, Germany) for 16 h. After transduction, Smyle/pp65 DC were washed twice with phosphate-buffered saline (PBS) and further maintained in culture with serum-free Cellgro medium. For production of conventional IFN-α-DCs monocytes were incubated with ID-LV-pp65 as described above. Following 16 h transduction, LV was removed and cells were maintained in culture in the presence of recombinant human GM-CSF (50 ng/ml), and IFN-α (1000 U/ml, PBL InterferonSource, New Jersey, USA). Cytokines for Con-IFN/pp65 were replenished every 3 days, while SmyleDC were incubated without cytokines in the medium. iDC were harvested after 7, 14 and 21 days of culture. For mouse immunizations, Smyle/pp65 at day 1 or Con-IFN/pp65 DC at day 7 after transduction were resuspended in PBS, used for s.c. injection. The number of viable counts was determined with trypan blue exclusion.

Mouse transplantation with human HSC

NOD.Cg-*Rag1*^{tm1Mom} *Il2rg*^{tm1Wjl} (NOD;$Rag1^{-/-}$;IL-$2r\gamma^{-/-}$, NRG) mice were bred and maintained under pathogen free conditions in an IVC system (BioZone, United Kingdom). All procedures involving mice were reviewed and approved by the Lower Saxony and followed the guidelines provided by the Animal Facility at Hannover Medical School. For HSC transplantation, 4-week old mice were sublethally irradiated (450 cGy) using a ^{137}Cs column irradiator (gammacell, company, country). Mouse recipients were intravenously injected with $5x10^5$ human CD34+ peripheral blood HSC into the tail vein. Mice were bled at different time points (6, 10 and 13) after human HSC transplantation to monitor the status of human hematopoietic cell engraftment and were sacrificed at week 20 for final analyses. For DC immunizations, Smyle/pp65 or Con-IFN/pp65 DC were collected from culture plates and resuspended at a concentration of $5x10^5$ cells in 100 µL of PBS. HSC-reconstituted mice were injected at 10 and 11 week after HSC transplantation with DC suspensions by subcutaneously injection into the mouse right hind limb using a 27-gauge needle.

Flow cytometry analysis

Engraftment of human hematopoietic cells in human HSC-reconstituted mice was evaluated in peripheral blood and spleens using the following mouse anti-human antibodies: PerCP anti-CD45, Alexa700 anti-CD19, Pacific blue anti-CD4, APC anti-CD3, PE-Cy7 anti-CD8, FITC anti-CD45RA,

PE anti-CD62L (Biolegend), PE anti-CD14, FITC anti-Lineage positive, APC anti-CD11c, PE anti-CD123 (Becton Dickinson). For peripheral blood analyses, blood was lysed by two rounds of incubation with erythrocyte lysis buffer (0.83% ammonium chloride/20mMHepes, pH 7.2) for 5 min at room temperature followed by stabilization with cold phosphate buffered saline (PBS) and centrifugation for 5 min at 300g. Cells were incubated with antibodies for 30 min at 4°C. Harvested spleen cells were treated with erythrocyte lysis buffer (0.83% ammonium chloride/20mMHepes, pH 7.2) for 5 min, washed with phosphate buffered saline (PBS) and incubated with antibodies for 30 min on ice. After a washing step, cells were resuspended in PBS and acquired in LSR flow cytometer (Becton Dickinson). For DC phenotypic characterization the following anti-human antibodies were used: PE anti-CD80, PerCP anti-HLA-DR, APC anti-CD86, APC anti-CD83 (Becton Dickinson). For DC staining, cells were collected, washed once with PBS and incubated with mouse IgG (50 µg/mL) on ice for 15 min followed by incubation with the antibodies. Cells were washed, resuspended in cell fix solution (Becton Dickinson) and further analyzed using a FACSCalibur cytometer. Analyses were performed using FlowJo software (Tree Star, Inc.).

Histology and immunohistochemistry analysis of human T cell engraftment

LN from human HSC-reconstituted NRG or C57Bl6 wild type mice were harvested and embedded in optimal cutting temperature compound (O.C.T. Sakura Finetek, Torrance, CA, USA) for cryopreservation. Frozen sections (5µm) were fixed by acetone and stained with monoclonal anti-mouse or human CD3 (eBioscience, San Diego, CA, USA), anti mouse or human CD11c (eBioscience), anti-mouse LYVE-1 (Dako), anti-CD31 mouse (BD Bioscience). Immunofluorescence analyses were performed in a AXIOCAM fluorescence microscope (Zeiss).

In vivo bio-luminescence imaging analyses

Mice were anesthetized with ketamine (100 mg/kg intraperitoneally) and xylazine (10 mg/kg intraperitoneally), and an aqueous solution of d-luciferin (150 mg/kg intraperitoneally) was injected 5 minutes before imaging. Mice were placed into a dark chamber of the charge-coupled device camera (IVIS 200, Xenogen, Cranbury, NJ, USA), and grayscale body surface reference images (digital photograph) were taken under weak illumination. After the light source was switched off, photons emitted from luciferase-expressing cells within the animal body and transmitted through the tissue were quantified over a defined time of up to 5 minutes using the software program Living Image (Xenogen) as an overlay on Igor (Wavemetrics, Seattle, WA, USA). For anatomical localization, a pseudocolor image representing light intensity (blue, least intense; red, most intense) was generated in Living Image and superimposed over the grayscale reference image. Quantified luminescence consists in averaged photon radiance on the surface of the animal and is expressed as photons/sec/cm^2/sr where sr=steradian.

Functional analyses of pp65-CTLs recovered from mouse LN and spleen

For evaluation of immune responses against CMV-pp65, splenocytes from each group were harvested, pooled stained with APC-conjugated anti-human CD3 and sorted using a XDP cell sorter (Beckman Coulter). Human $CD3^+$ cells were seeded at a density of 10.000 cells per well in anti-human IFN-γ-coated 96-well ELISPOT plate and incubated overnight in the presence of 10 µg/mL of pp65 overlapping peptide pool (Miltenyi). CEF recall peptide pool corresponding to a mixture of CMV, Epstein-Barr virus and influenza virus epitopes (PANA Tecs GmbH, Tuebingen, Germany) was used as positive control. Next day, cells were washed and plates were further incubated with biotin-conjugated anti-human IFN-γ antibodies followed by alkaline phosphatase-conjugated streptavidine. Plates were developed using NBT/BCIP liquid substrate and analyzed in an AELVIS ELISPOT reader (AELVIS GmbH, Hannover, Germany). For analyses of lymphocytes obtained from LN, cells were expanded *ex vivo* for seven days in the presence of SmyleDC or SmyleDC/pp65 and exposed to pp65 overlapping peptide pool on a ELISPT plate and IFN-γ spots were counted.

Immunoglobulin production in HSC-NRG mice

Plasma was harvested from HSC-NRG mice 20 weeks after reconstitution (8 weeks after second Smyle or IFN-conDC) and screened by ELISA for the presence of total human IgM an total human IgG as described elsewhere (Becker, P. D. et al., 2010, "Generation of human antigen-specific monoclonal IgM antibodies using vaccinated "human immune system" mice" *PLoS One* 5). Total IgM and IgG determination was performed by coating 96-well plates either with AffiniPure $F(ab')_2$ fragment goat anti-human IgM (Fc5µ-specific, Jackson ImmunoResearch) or AffiniPure goat anti-human IgG (Fcγ fragment-specific; Jackson ImmunoResearch). Control human serum protein calibrator (Dako) with known IgM (0.8 mg/ml) and IgG (10.4 mg/ml) concentrations was used as a standard to be compared to the samples. After coating, the plates were washed in ELISA wash buffer (PBS, 0.5% Tween-20), blocked with 4% of milk and further incubated with serial dilution of mouse plasma (starting at a dilution of 1:5). Enzyme-conjugated detection antibodies were added at a dilution of 1:2500 for HRP-conjugated anti-IgG and a dilution of 1:5000 for HRP-conjugated anti-IgM (both from Jackson ImmunoResearch). TMB substrate/stop solution (Biosource) was used for the development of the ELISA assay.

Statistical Analysis

Parametric (*t* test) and non-parametric (Kruskall-Wallis) statistical analyses were performed to compare the differences among groups for engrarftment of hematopoietic lineages in HIS-NRG mice. Analyses were performed in Graph prism 5^{th} version software. All tests were two-sided, and $P < 0.05$ was considered significant.

Results

LV-induced Smyle/pp65 DC generation and characterization

We have recently shown that integrase-defective (ID)-LV used to promote constitutive expression of human GM-CSF and IFNα in human monocytes induced highly viable IFNα-DC with high activating

status and high viability and engraftment properties in vivo (Daenthanasanmak, A. et al., 2012, "Integrase-defective lentiviral vectors encoding cytokines induce differentiation of human dendritic cells and stimulate multivalent immune responses in vitro and in vivo" *Vaccine* 30: 5118-5131). These LV-induced DC, named as "Smyle" (Self-differentiated, myeloid-derived, lentivirus-induced) DC, could be additionally co-transduced with a ID-LV for expression of the CMV tegument viral protein pp65. Smyle/pp65 potently stimulated anti CMV-specific CTL responses *in vitro* and *in vivo*. Here, we aimed to test the feasibility of Smyle/pp65 DC generation using leukapheresis obtained from GCSF-mobilized hematopoietic stem cell donors (**Fig 1A**). Briefly, for Smyle/pp65 generation, $CD14^+$ cells were isolated by magnetic selection of PBMC obtained from GCSF-mobilized HSCT donor leukapheresis and preconditioned with GM-CSF and IL-4 followed by overnight LV co-transduction with bicistronic LV expressing GM-CSF and IFN-α and LV expressing CMV-pp65. After LV removal, Smyle/pp65 were maintained in culture without cytokine supplement. Conventional IFNα_DC expressing pp65 (Con-IFN/pp65) were produced with monoytes similarly transduced with LV-pp65 and maintained in culture supplemented every third day with recombinant human GM-CSF and IFNα. Cells were cultured for up to three weeks to determine their differentiation status, viability and phenotype stability. We were able to recover comparable levels of Con-IFN/pp65 and Smyle/pp65 DC (**45 vs. 35.6%, p>0.05**) at day 7 of culture (**Fig 1B**). Importantly, Smyle/pp65 showed 3-fold higher levels of recovery than Con-IFN already at day 14 of culture (35.4 vs. 14.2% p=0.021). Three weeks after DC culture, both Smyle/pp65 and Con-IFN/pp65 significantly lost viability, yet Smyle/pp65 showed higher levels compared to Con-IFN/pp65 (17 vs. 5%, $p<0.05$). We also evaluated the differentiation status of Smyle/pp65 and Con-IFN/5pp65 throughout the culture period. Co-expression of pp65 was confirmed by intracellular staining and flow cytometry analyses. Levels of CMV-pp65 expression were maintained higher in Smyle/pp65 than in Con-IFN/pp65 DC (55 vs. 21.2%, p=0.014) (**Fig 1C**). On day 7 of culture, both Smyle/pp65 and Con-IFN/pp65 displayed typical DC differentiated phenotype, characterized by high expression levels of CD11c, CD86 and MHC-II (HLA-DR) (**Fig 1D**). Both cell types presented comparable maturation status, as shown by CD80 and CD83 expression. Smyle/pp65 maintained a stable expression of immunophenotypic markers at longer culture periods of 14 and 21 days. Despite culture in the presence of recombinant cytokines, Con-IFN/pp65 DC de-differentiated, losing the expression of differentiation and maturation markers.

Both Smyle/pp65 and Con-IFN/pp65 maintained in culture secreted several endogenously up-regulated cytokines, that accumulated in the culture supernatants and were detectable by cytokine array analyses: IFN-g, IL-10, IL-12, 1L-13, IL-1β, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, MCP-1 and TNF-α showed an overall enhanced activation of Con-IFN/pp65 (**Fig. 2**). Accumulated levels of IL-1β, 4, 6, 8, 12 were higher for Con-IFN/pp65 cultures, which implies that, although these cells were continuously exposed to high levels of several cytokines, their functionality in terms of maintaining expression of relevant immunophenotypic markers was reduced.

Smyle/pp65 supports recovery of lymphocyte compartment after human HSC transplantation

In order to evaluate the potential of Smyle/pp65 to induce immune-reconstitution in a HSC transplantation setting, we first established a humanized immune system model of (HIS) by transferring human $CD34^+$ cells into four-week old, sub-lethally irradiated NOD.$Rag1^{-/-}$.$IL2r\gamma^{-/-}$ (NRG) mice. We detected $CD3^+$ human T cells in peripheral blood already at six weeks post HSCT (0.35%), reaching average frequencies of 8.6% twenty weeks after $CD34^+$ HSC transfer (data not shown). Human $CD19^+$ B cells predominated within detectable human $CD45^+$ cells, with levels ranging from 84% (week 6) up to 77% (week 20) (data not shown). 20 weeks after HSC reconstitution of HIS-NRG, human $CD45^+$ cells corresponded to 3.9% of total splenocytes and $CD19^+$ B cells represented to the majority of the human cell content (84%). Human $CD3^+$ T lymphocytes corresponded to 7.8% of human CD45-expressing cells and contained $CD4^+$ and $CD8^+$ at a ratio of 1:1 (data not shown).

We next assessed whether immunization with Smyle/pp65 improved immune reconstitution in HIS-NRG mice. We followed a prime/boost immunization scheme consisting in one injection of DC in week 10 after HSCT followed by a boost injection one week later. Immunizations were performed by subcutaneous injections of Smyle/pp65 harvested immediately after LV-transduction or 7 days-cultured Con-IFN/pp65. DC cell suspensions ($5x10^5$) were injected into the right flank, as previously described (Salguero, G. et al., 2011, "Preconditioning therapy with lentiviral vector-programmed dendritic cells accelerates the homeostatic expansion of antigen-reactive human T cells in NOD.Rag1-/-.IL-2rgammac-/- mice" *Hum Gene Ther* 22: 1209-1224). Non-immunized mice served as controls (**Fig 3A**). We first evaluated the effect of DC injections on the reconstitution of the human $CD45^+$ cells in peripheral blood. Frequencies of human $CD45^+$ were similar in all groups before immunization at week 10. One week after prime/boost immunization, mice immunized with Smyle/pp65 showed significantly enhanced levels of human $CD45^+$ cells as compared with non-immunized controls (1.7% vs. 0.64%, p=0.01). $CD45^+$ cell frequencies were not significantly higher in Con-IFN/pp65-immunized mice (1.6% compared to controls, p=0.09). Importantly, significant enhanced levels of $CD45^+$ were maintained 8 weeks after Smyle/pp65 immunization compared with mouse controls (1.9% vs. 0.2%, p=0.03). Mice vaccinated with Con-IFN/pp65 also showed higher but no significant levels of $CD45^+$ cells in blood (1.3% vs, 0.2%, p=0.08). We next analyzed the T cell compartment after DC immunization. Smyle/pp65 immunization led to early significant increase of $CD3^+$ frequency in peripheral blood compared to control mice (0.16% vs. 0.03%, $p<0.04$) and supported long term engraftment of human T cells compared with controls (1.8% vs. 0.03%, p=0.04) 20 weeks after HSCT. (**Fig. 3B**). Remarkably, Con-IFN/pp65-immunization did not induce neither early, nor long term increased levels of human $CD3^+$ T cells in HIS-NRG mice (0.15%, p=0.26 +1 week; 0.37%, p=0.31 +8 weeks after DC injection, compared to controls). Analysis of the relative frequency of human T lymphocytes among $CD45^+$ cells 8 week after DC immunization, showed significant enhanced frequency of $CD3^+$ compartment in Smyle/pp65 immunized mice compared to controls (59.7% vs. 8.6%, p=0.0001) and Con-IFN/pp65 (59.7% vs. 21.7%, p=0.001) and confirmed that long term

engraftment of human $CD45^+$ cells was determined by expansion of the human T cell compartment in these mice.

We further analyzed the cell content of spleens from vaccinated and control mice 20 week after HSC reconstitution (**Fig. 4**). Smyle/pp65 immunized mice showed significantly higher engraftment levels of human $CD45^+$ compared to non-immunized (19.1% vs. 3.1%, p=0.007) and Con-IFN/pp65-immunized (19.1% vs. 5.9%, p=0.01) mice. Accordingly, higher frequencies of human $CD3^+$ cells were observed in Smyle/pp65 immunized mice as compared to control mice (10.1% vs. 0.31%, p=0.007), corresponding to 40.8% of total human $CD45^+$ cells (**Fig 4A**). Con-IFN/pp65 immunization failed to enhance the frequency of $CD3^+$ cells (0.17%, p=0.5 vs. control), corresponding to only 3.9% of $CD45^+$ cells in spleen. Distribution of lymphocyte subsets within $CD3^+$ T cells were further analyzed in reconstituted NRG mice spleens (**Fig. 4B**). Although we did not observe significant differences among $CD3^+/CD8^+$ cells in the three groups (control, 51%; Con-IFN/pp65 40.9%; Smyle/pp65 44.2%, p>0.05), we found significant reduced levels of $CD8^+/CD45RA^+/CD62L^+$ naïve cells in Smyle/pp65-immunized spleenocytes, compared to non-immunized controls (12.3% vs. 37.49%, p=0,03). Conversely, frequencies of $CD8^+/CD45RA^-CD62L^-$ effector memory T cells in Smyle/pp65 were significantly higher than control NRG mice (38.5% vs. 19.5%, p=0.04). Similar but not significant distribution of $CD8^+$ T cells subsets was found in mice injected with Con-IFN/pp65 DCs for Naïve (14.5%) and effector memory (24.7%) populations. Analysis of $CD3^+/CD4^+$ frequencies did not show statistical differences among mouse groups for total $CD4^+$ T cells (control, 44.7%; Con-IFN/pp65 34.7%; Smyle/pp65 50%, p>0.05). Nevertheless, reduction in Naïve and increase in effector memory T cells due to Con-IFN/pp65 and Smyle/pp65 immunizations compared to controls were also seen but were not significant. Taken these data together, human Smyle/pp65 immunization after HSCT promoted a rapid and sustained reconstitution of the T cell compartment and significantly favoured the expansion of $CD8^+$ T -and in less extent $CD4^+$, with a predominantly effector memory phenotype.

Smyle/pp65 immunization induces reconstitution of peripheral lymph nodes.

We next analyzed mice injected with Smyle/pp65 or Con-IFN/pp65 DCs for the presence of lymph nodes (LN) 20 weeks after HSC reconstitution. We detected a high frequency of LN formation in mice injected with Smyle/pp65 (65%), whereas control mice or mice injected with Con-IFN/pp65 showed low occurrence of LN structures (11% and 28%, respectively) (**Fig. 5A**). Quantification of the frequency of LN in different regions of be animal body revealed a strong correlation between the DC injection site and the formation of LN at the corresponding draining site (**Fig. 5B**). Inguinal (57%), iliac (35%) and axillary (56%) LNs were observed in mice immunized with Smyle/pp65, compared to complete absence of LN in control mice at the same side. Importantly, Con-IFN/pp65 injection did not induce iliac LN formation and only induced formation of inguinal and axillary LN in 14% and 28% mice, respectively.

We next performed immunohistological analyses of LN obtained from Smyle/pp65-imunized NRG mice. LN architecture in LN from reconstituted NRG showed lack of B cell follicles compared to normal wild type LNs obtained from wild type C57BL/6 (**Fig 6**). Humanized LN were predominantly populated by human $CD3^+$ T cells and we also observed the presence of human DC (CD11c+). LN were encapsulated by a layer of cells positive for mouse lymphatic vascular cell (LYVE-1) and mouse endothelial vascular CD31 marker. Importantly, we also observed the presence of structures resembling high endothelial venules (HEV) that were positive for mouse CD31, suggesting a rudimentary vascular organization process within the forming LN.

We next evaluated whether injected Smyle/pp65 DC were able to migrate to the reconstituted LN formed in HIS-NRG mice. Smyle/pp65 were co-transduced with a LV expressing firefly luciferase (LV-fLUC), such that they could produce bioluminescence upon exposure to Luciferin. Smyle/pp65-fLUC were injected into the hind limb of HSC-NRG mice 6 weeks after immunization at the right side, where LNs were more frequently found (**Fig 7**). As a control for DC migration, we injected fLUC-Smyle/pp65 in the contralateral flank. Engraftment and migration of fLUC-Smyle/pp65 was followed weekly by *in vivo* bioluminescence imaging. We found accumulation of bioluminescence signal at the LN position in the injection side on day 21 after DC injection as compared to the same location in the contralateral flank. Furthermore, when mice were euthanized and LN were exposed, Smyle/pp65 luminescence was located in the formed inguinal LN, the ipsilateral axillary LN but not intraabdominal LN such as mesenteric (**Fig 7**). This data indicates that Smyle/pp65 DC are able to migrate to sites were regional draining LN Anlage are located, and trigger LN formation.

Smyle/pp65 induces specific immune responses in HIS-NRG mice

We have previously demonstrated that Smyle/pp65 stimulates anti-pp65 specific responses in a peripheral blood lymphocyte (PBL) mouse model (Daenthanasanmak, A. et al., 2012, "Integrase-defective lentiviral vectors encoding cytokines induce differentiation of human dendritic cells and stimulate multivalent immune responses in vitro and in vivo." *Vaccine* 30: 5118-5131). Here we evaluate whether Smyle/pp65 immunization reconstituted NRG mice could stimulate specific T cell responses against CMV-pp65. Since we observed a significant effect of Smyle/pp65 in LN formation, we first wanted to test if these findings correlated with enhanced antigen specific reactivity against CMV-pp65 in local LN. We first evaluated the cell content of reconstituted LN after Smyle/pp65 immunization by flow cytometry. The majority of LN cells were human $CD45^+$ (77%), with 73% corresponding to $CD3^+$ T lymphocytes and 3.8% corresponding to $CD19^+$ B cells (**Fig 8A**). Among human $CD3^+$ cells we found that 42% were $CD4^+$ and 56% were $CD8^+$ 56%, with a predominance of effector memory phenotype for both T cell subsets (80% and 76% respectively) (**Fig. 8B**). In order to measure CMV-pp65 specific responses, LN cells were isolated 8 weeks after immunization and ex-vivo expanded in the presence of Smyle/pp65 DC for 7 days. SmyleDC not expressing the CMV-pp65 antigen served as controls (**Fig 8C**). After DC co-culture, cells were collected and seeded in IFN-γ-coated plates, re-stimulated with CMV-pp65 overlapping pool peptide and analyzed by ELISPOT for IFN-γ production. PBMNC from CMV-reactive healthy donor were used as positive control for IFN-γ

production. Remarkably, LN cells showed significant reactivity against CMV-pp65 after ex-vivo expansion as compared with LN cells in the presence of Smyle DC with out antigen (53 vs. 18.7 spots, $p<0.021$, n= 5 mouse donors) (**Fig 8C**). In addition, we evaluated systemic specific immune responses against CMV, by recovering human $CD3^+$ T cells from spleens of control, Smyle/pp65 and Con-IFN/pp65-immunized NRG mice (**Fig. 8D**). We first promoted T cell proliferation, by incubation for 48 h with human anti-CD2, anti-CD3 and anti-CD28 beads in the presence of human recombinant IL-7 and IL-15 followed by co-culture with Smyle/pp65 DC for additional 7 days in the presence of IL7/IL15. Cells co-cultured with Smyle DC lacking the expression of CMV-pp65 served as controls. T cells recovered from spleens of HSC-NRG mice immunized with Smyle/pp65 and further expanded with Smyle/pp65 showed significant increased of averaged of positive spots compared to controls (33.6 spots vs. 0.5, $p<0.05$) (**Fig 8D**). Conversely, T cells recovered from spleens of Conv-IFN/pp65-injected mice had reduced CMV-pp65 (15.5 averaged spots, $p>0.05$ vs. Smyle/pp65).

Immunoglobulin production in HSC-NRG mice

We characterized the B lymphocyte compartment in reconstituted NRG mice DC after immunization. Frequency of $CD19^+$ B lymphocytes (this is a relatively early B cell population) was not significantly different among all groups previous immunization (1.6%, 4.5% and 2% for control, Con-IFN/pp65 and Smyle/pp65 respectively) (**Fig 9A**). One week after second DC immunization overall levels of $CD19^+$ B cells were decreased, however Smyle/pp65-injected NRG mice showed higher frequencies of B cells as compared with control and Con-IFN/pp65-injected mice (1.1% vs. 0.4% and 0.9% respectively, $p=0.02$). By week eight post immunization, overall frequencies of B cell were bellow 1% in all groups (control 0.18%; Con-IFN/pp65 0.1%; Smyle/pp65 0.37%). We were also able to recover B cells in spleens eight weeks post-immunization and observed non-significant differences of human $CD19^+$ cells among controls (2.5%), Con-IFN/pp65 (5.1%) and Smyle/pp65-immunized (7.8%) NRG mice. In order to evaluate the functionality of human B cells in reconstituted mice, we further measured immunoglobulin (Ig) G and M concentration in plasma from NRG mice eight weeks after DC immunization. Remarkably, we found significantly higher levels of IgG in Smyle/pp65-injected mice (59.6 µg/mL) compared with almost undetectable levels in control (0.78 µg/mL) and Con-IFN/pp65-immunized (0.047 µg/mL) mice. Similarly, IgM concentration was higher in plasma from Smyle/pp65-injected mice (26.6%) compared to control and Con-IFN/pp65 (0.15 and 0.01 µg/mL, respectively).

Discussion

DC are pivotal for "organizing" the development of LN, which are the most effective site for stimulation of adaptive T and B cell immune responses. Using a modality of iDC (IDLV-SmyleDC/pp65) described above, we evaluated the effects of DC vaccination in an immunodeficient mouse strain transplanted with human HSC. Lymphopenic mouse models making use of transplanted human hematopoieitic stem cell precursors/ stem cells (such as

$CD34^+$ cells) have been developed worldwide in order to reconstitute the human immune system in mice (Lepus CM et al. "Comparison of Human Fetal Liver, Umbilical Cord Blood, and Adult Blood Hematopoietic Stem Cell Engraftment in NOD-scid/γc−/−, Balb/c-$Rag1^{-/-}\gamma c^{-/-}$, and C.B-17-scid/bg Immunodeficient Mice". *Human immunology.* Oct 2009;70(10):790-802). These models have been explored to follow several steps of hematologic reconstitution such as HSC engraftment in bone marrow niches, mobilization, self-renewal, differentiation in several lineages. Long-term (16-20 weeks) follow-up of these mice after HSCT showed a generally impaired $CD8^+$ T cell maintenance (Andre MC et al. "Long-term human CD34+ stem cell-engrafted nonobese diabetic/SCID/IL-2R gamma(null) mice show impaired CD8+ T cell maintenance and a functional arrest of immature NK cells". *J Immunol.* Sep 1 2010;185(5):2710-2720.). Mice transplanted with human HSC did not develop regenerated LN containing viable and functional T cells. Lymph nodes are the specialized tissues where the drained lymph is "filtered" for immune surveillance of pathogenic conditions (such as infections, cancer). Due to its specialized architecture, lymph nodes allow optimization of antigen presentation to T cells for priming and amplification of adaptive immune responses. Demonstration of antigen-specific CTL responses generated from LN in humanized mice have not been described with the previously available approaches such as exploring transgenic expression of human cytokines that are critical for adaptive immune responses (for example IL-7, IL-15, GMCSF) or by transgenic approaches of single human MHC class I or II molecules. On the other hand, the iDC immunization approach described here brings together into the immune deficient host a highly viable human professional antigen presenting cell perfectly matched with all the MHC molecules expressed by human stem cell graft that expresses a combination of several human cytokines and a highly immunogenic antigen (known to stimulate several different MHC-restricted immune responses). Thus, based on these properties, SmyleDC/pp65 immunization produced a dramatic increase in the absolute frequency of human T cells circulating in the peripheral blood, CTL responses against the pp65 CMV viral antigen and high levels of human IgG in the plasma demonstrating that adaptive human immune responses in the mice have been regenerated.

Moreover, the ability of the iDC to promote regeneration of lymph nodes concomitantly with stimulation of adaptive T and B cell immune responses in immunodeficient mice reconstituted with human HSC indicates that iDC have properties that support a general regeneration of a functional immune system from transplanted human HSC. Thus, iDC may be used in human patients who were transplanted with HSC in order to accelerate the development of a fully functional immune system, thus decreasing the susceptibility to infectious diseases or relapse of the malignancy after HSC transplantation.

.

All together, the data describe above demonstrated:

1. The fundamental difference between conventional human DC vaccines (which are not highly viable or immunologically stable *in vitro* or *in vivo*) with our iDC (viable and potent *in vitro* and *in vivo* for several weeks);

2. The capacity of iDC once injected subcutaneously to trafficking to the LN-"Anlage", resulting in recruitment of CD4+ and CD8+ cells (particularly represented by effector memory and central memory cells);

3. That iDC administration resulted in adaptive CTL responses measurable as antigen-specific responses against a protein expressed in CMV (pp65);

4. The effects of iDC immunization to stimulate high levels of human IgG production in mice, demonstrating that during the development of human B cells in NRG immunoglobulin class switch occurred;

5. Applicability of safety enhanced integrase-defective lentivirus in iDC reprogramming, which enhances the safety of the genetic programming by lowering the risks of insertional mutagenesis;.

6. Realistic perspectives for clinical development and use of iDC, since: 1. Lentiviral vectors are already being used for ex vivo gene transfer in clinical gene therapy protocols, 2. The HSC source we employed is routinely used for clinical HSCT, 3. The pp65 CMV antigen employed is of clinical relevance for stimulation of potent anti-CMV responses in lymphopenic hosts after HSCT.

Claims

1. An induced dendritic cell (iDC) engineered to express

   a) at least one cytokine which induces the self-differentiation of human dendritic cell (DC) progenitor cells into DCs; and

   b) at least one antigen;

   for use as a medicament.

2. An iDC engineered to express

   a) at least one cytokine which induces the self-differentiation of human dendritic cell (DC) progenitor cells into DCs; and

   b) at least one antigen;

   for use in the regeneration of the immune system of an immunodeficient subject following transplantation of hematopoietic stem cells (HSC).

3. The iDC according to claim 2, wherein the vector is a lentiviral vector.

4. The iDC according to claim 3, wherein the lentiviral vector is integrase defective.

5. The iDC according to any one of claims 2 to 4, wherein the iDC expressing at least one antigen expresses at least one cytokine which induces the self-differentiation of human dendritic cell (DC) progenitor cells into DCs.

6. The method according to claim 5, wherein the cytokine is selected from the group consisting of GM-CSF, IL-4, IFN-α, IL-15, TGF-B, TNF-α, FLT3L, IL-3 and CD40L.

7. The iDC according to claim 6, wherein the iDC expresses a combination of cytokines selected from the group consisting of (i) FLT3L and IL-3; (ii) FLT3L and CD40L; (iii) FLT3L and IFN α; (iv) GM-CSF and IFN- α and IL-15; (v) GM-CSF and IFN-α and TNF-α; and (vi) GM-CSF and IFN-α and TGF-B.

8. The iDC according to any one of claims 2 to 7, wherein one antigen is expressed by the iDC is an antigen which can induce a cytotoxic or humoral immune response selected from the group consisting of xeno-reactivity, allo-reactivity, neo-reactivity or auto-immunity

9. The iDC according to any one of claims 2 to 8 wherein the immunodeficiency of the subject is an immunodeficiency selected from the group consisting of immunodeficiency caused by ionizing radiation, immunodeficiency caused by the administration of at least one cytotoxic pharmaceutical, primary immunodeficiency and immunodeficiency caused by a pathogen.

10. The iDC according to any one of claims 2 to 9, wherein the hematopoietic stem cell transplant is autologous.

11. The iDC according to any one of claims 2 to 9, wherein the stem cell transplant is heterologous.

12. The iDC according to any one of claims 2 to 11, wherein the subject is human.

13. An iDC engineered to express

a) at least one cytokine which induces the self-differentiation of human dendritic cell (DC) progenitor cells into DCs; and

b) at least one antigen;

for use as a medicament for the treatment of cancer which spreads lymphatically or a disease caused by a lymphotrophic pathogen.

14. An iDC comprising at least one integrase-defective lentiviral vector, wherein said vector mediates expression of

a) at least one cytokine which induces the self-differentiation of human dendritic cell (DC) progenitor cells into DCs; and

b) at least one antigen.

15. A method for regenerating an immune system in an immunodeficient subject comprising the steps of

a) transplanting hematopoietic stem cells to the subject; and

b) administering to the subject an induced dendritic cell (iDC) engineered to express at least one antigen and at least one cytokine which induces the self-differentiation of human dendritic cell (DC) progenitor cells into DCs.

16. The method according to claim 15, wherein the subject is a mouse and the HSC are derived from a human.

17. The method according to claim 14, wherein the mouse is characterized by the presence of endogenous T-cells and endogenous progenitors of dendritic cells.

18. The method according to claim 16 or 17, wherein the mouse strain has a primary immune deficiency that leads to dysfunction or absence of adaptive immune system (including T and B cells).

19. The method according to any one of claims 16 to 18, wherein the mouse is selected from the group of strains consisting of NOD-Rag1^{null}IL2Ry^{null}-NRG, NOD/LtSz-SCID/IL2Ry^{null}-NSG and NOD/SCID/IL2Ry^{null}-NOG.

20. The method according to any one of claims 15 to 19, wherein the vector mediates the expression of the antigen pp65 and the cytokines (i) GM-CSF and (ii) interferon-α and/or interleukin-4.

21. A mouse with a regenerated immune system produced by a method according to any one of claims 16 to 20.

22. Use of the mouse according to claim 21 for the study of the human immune system or for the testing of drugs, implants or devices for their use in humans..

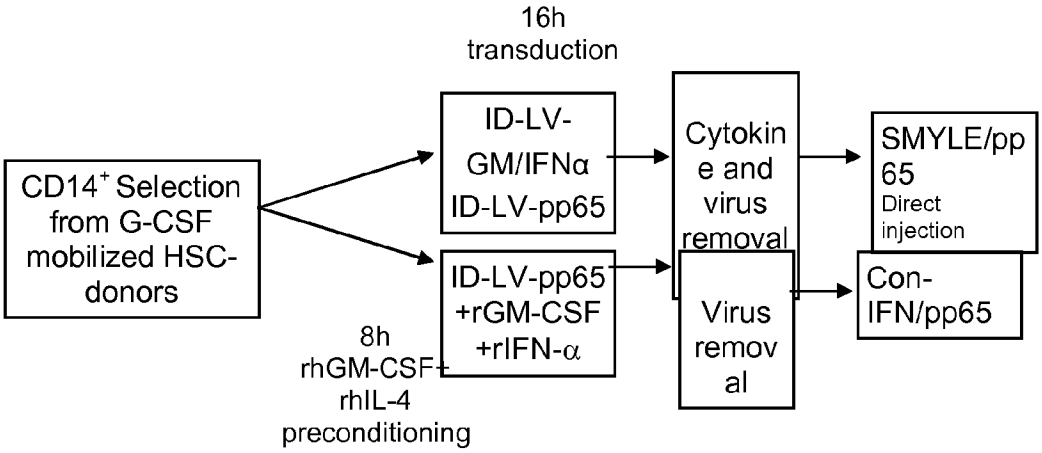

Figure 1A

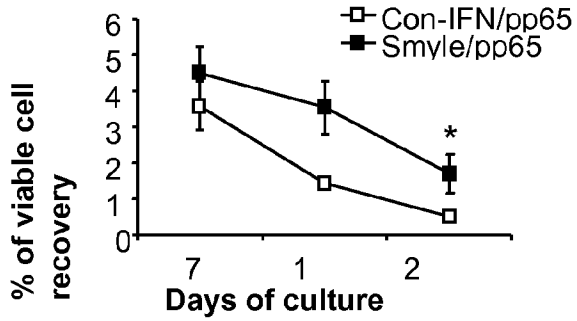

Figure 1B

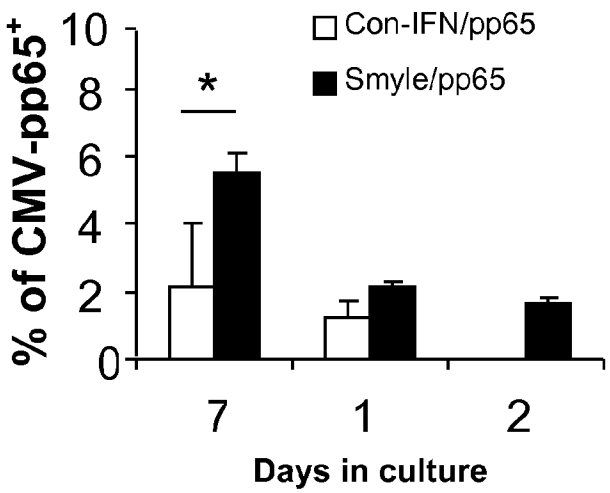

Figure 1C

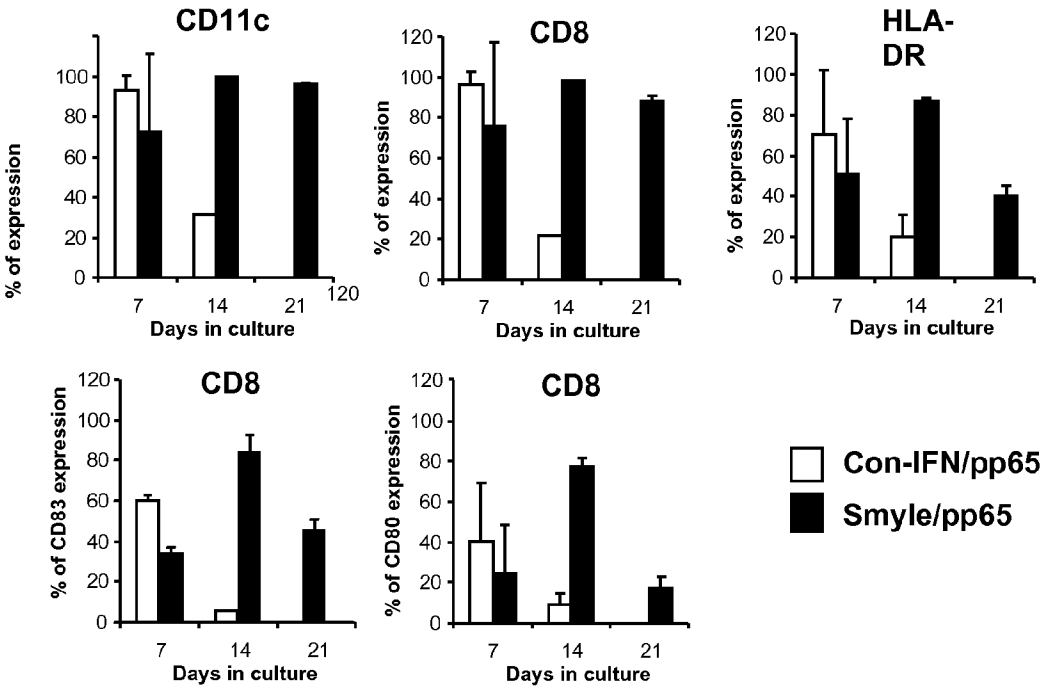

Figure 1D

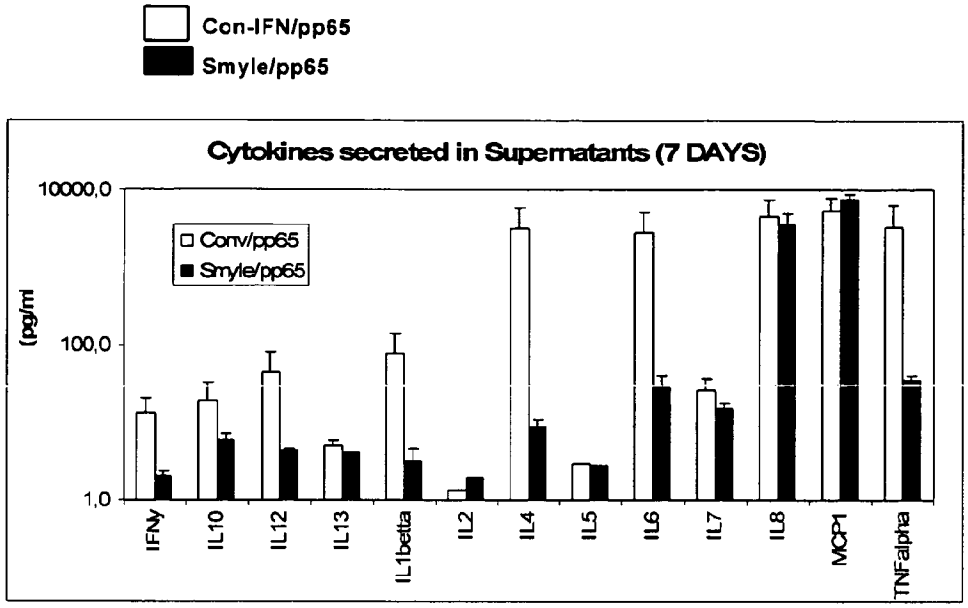

Figure 2A

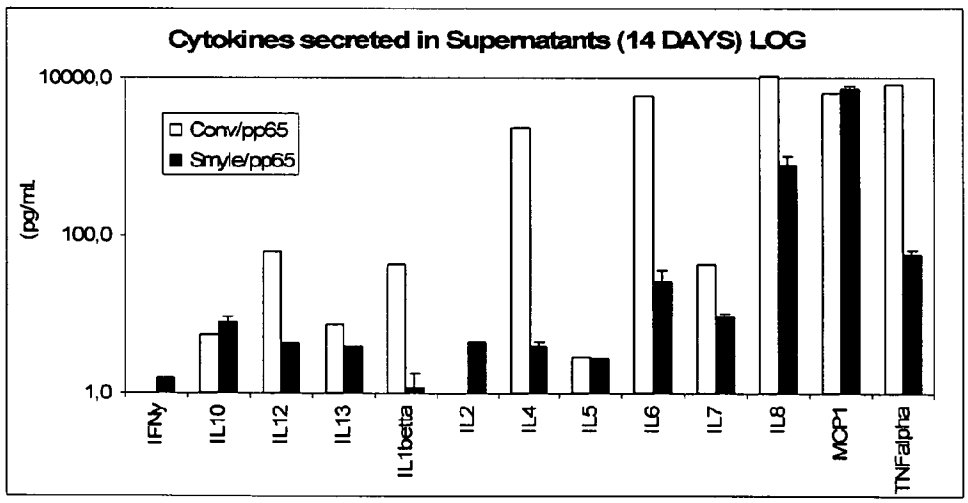

Figure 2B

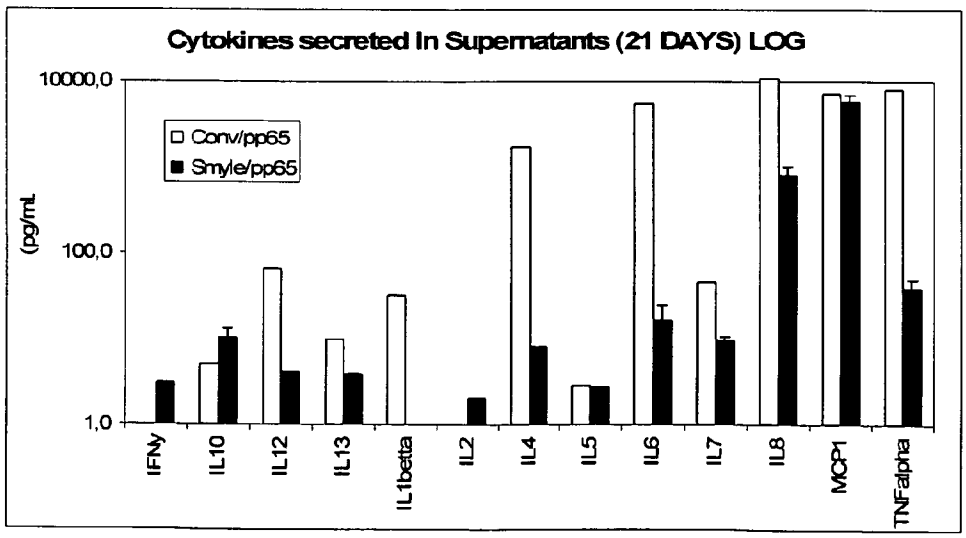

Figure 2C

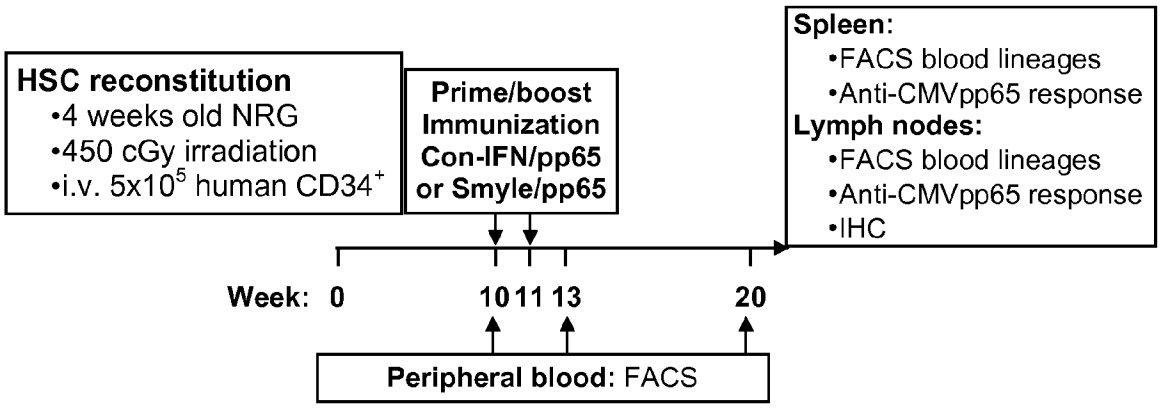

Figure 3A

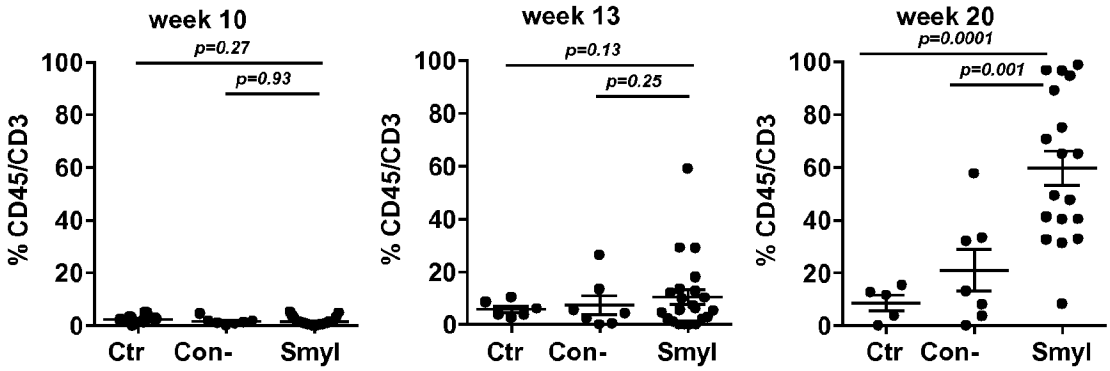

Figure 3B

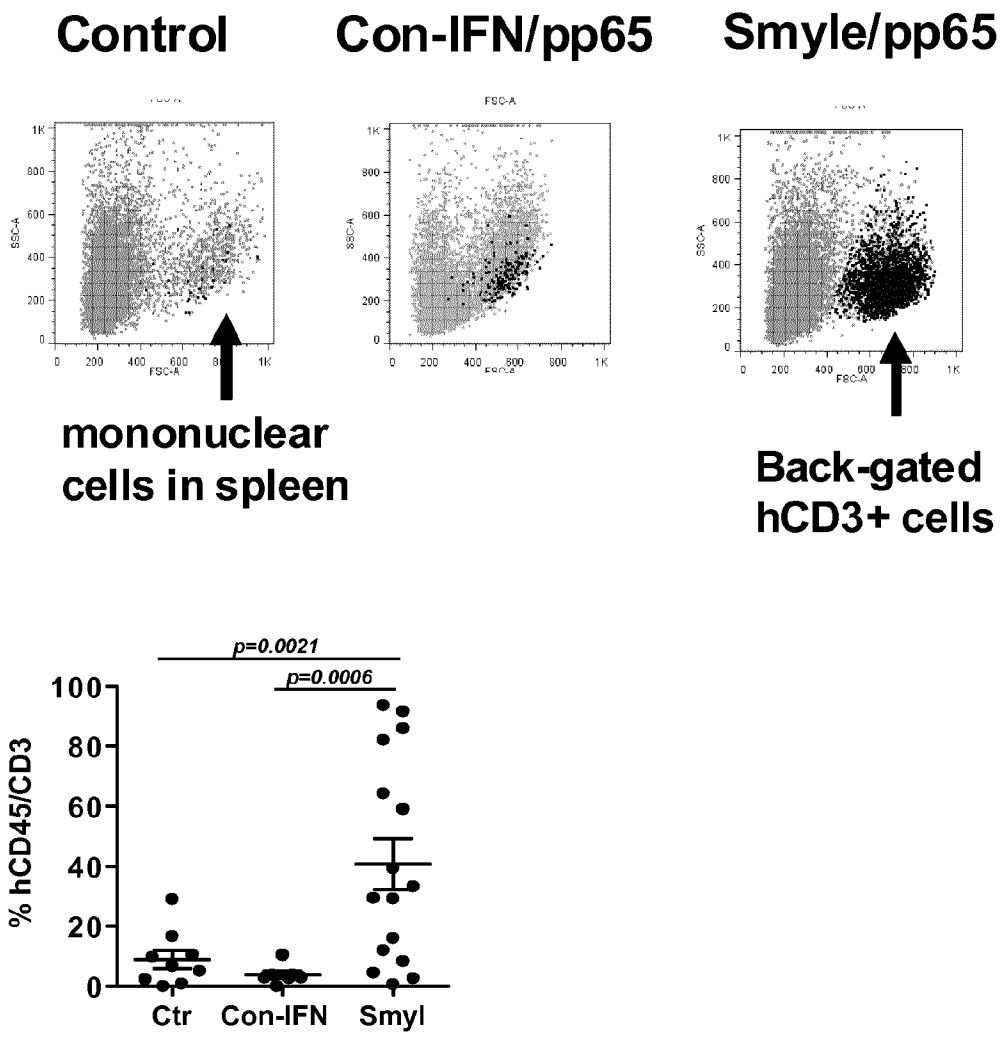
Control
Con-IFN/pp65
Smyle/pp65
mononuclear cells in spleen
Back-gated hCD3+ cells
p=0.0021
p=0.0006
% hCD45/CD3
100
80
60
40
20
0
Ctr
Con-IFN
Smyl

Figure 4A

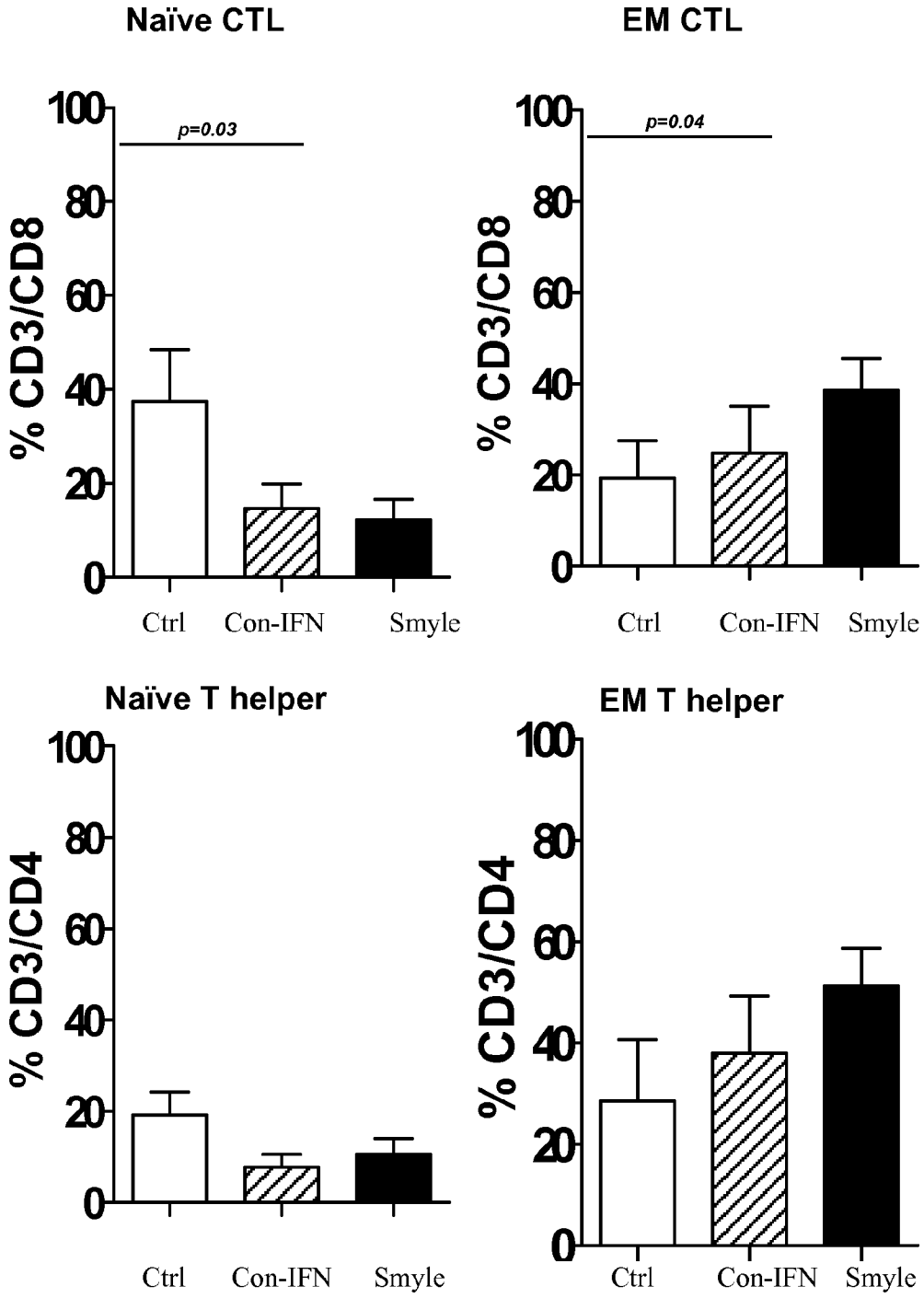
Naïve CTL
p=0.03
% CD3/CD8
100
80
60
40
20
0
Ctrl
Con-IFN
Smyle
EM CTL
p=0.04
% CD3/CD8
100
80
60
40
20
0
Ctrl
Con-IFN
Smyle
Naïve T helper
% CD3/CD4
100
80
60
40
20
0
Ctrl
Con-IFN
Smyle
EM T helper
% CD3/CD4
100
80
60
40
20
0
Ctrl
Con-IFN
Smyle

Figure 4B

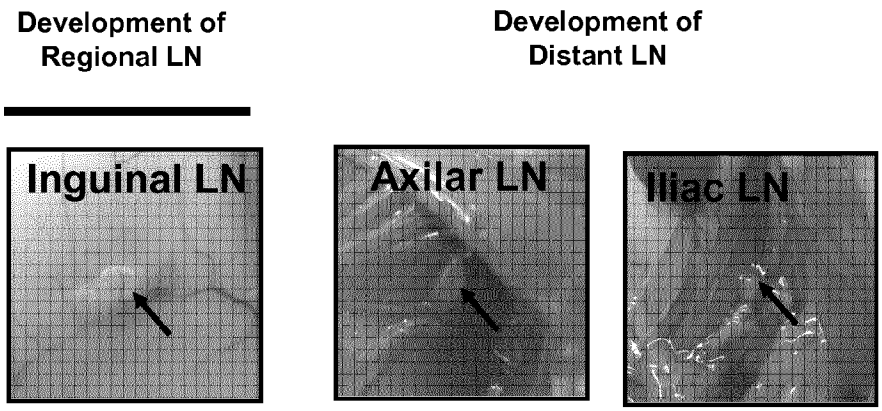
Development of Regional LN
Development of Distant LN
Inguinal LN
Axilar LN
Iliac LN

Figure 5

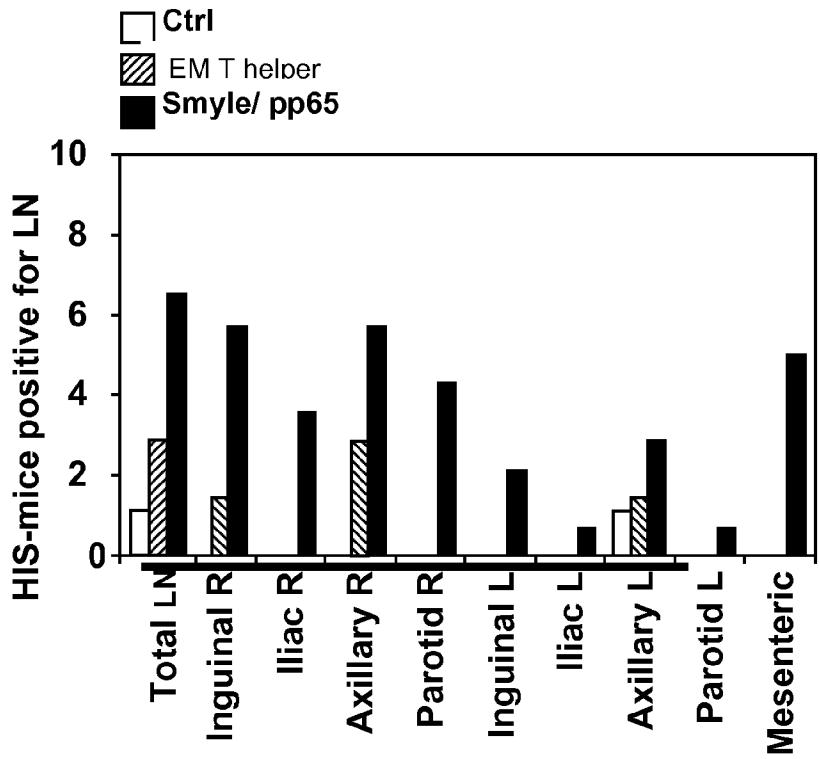
Ctrl
EM T helper
Smyle/ pp65
HIS-mice positive for LN
10
8
6
4
2
0
Total LN
Inguinal R
Iliac R
Axillary R
Parotid R
Inguinal L
Iliac L
Axillary L
Parotid L
Mesenteric

Figure 5B

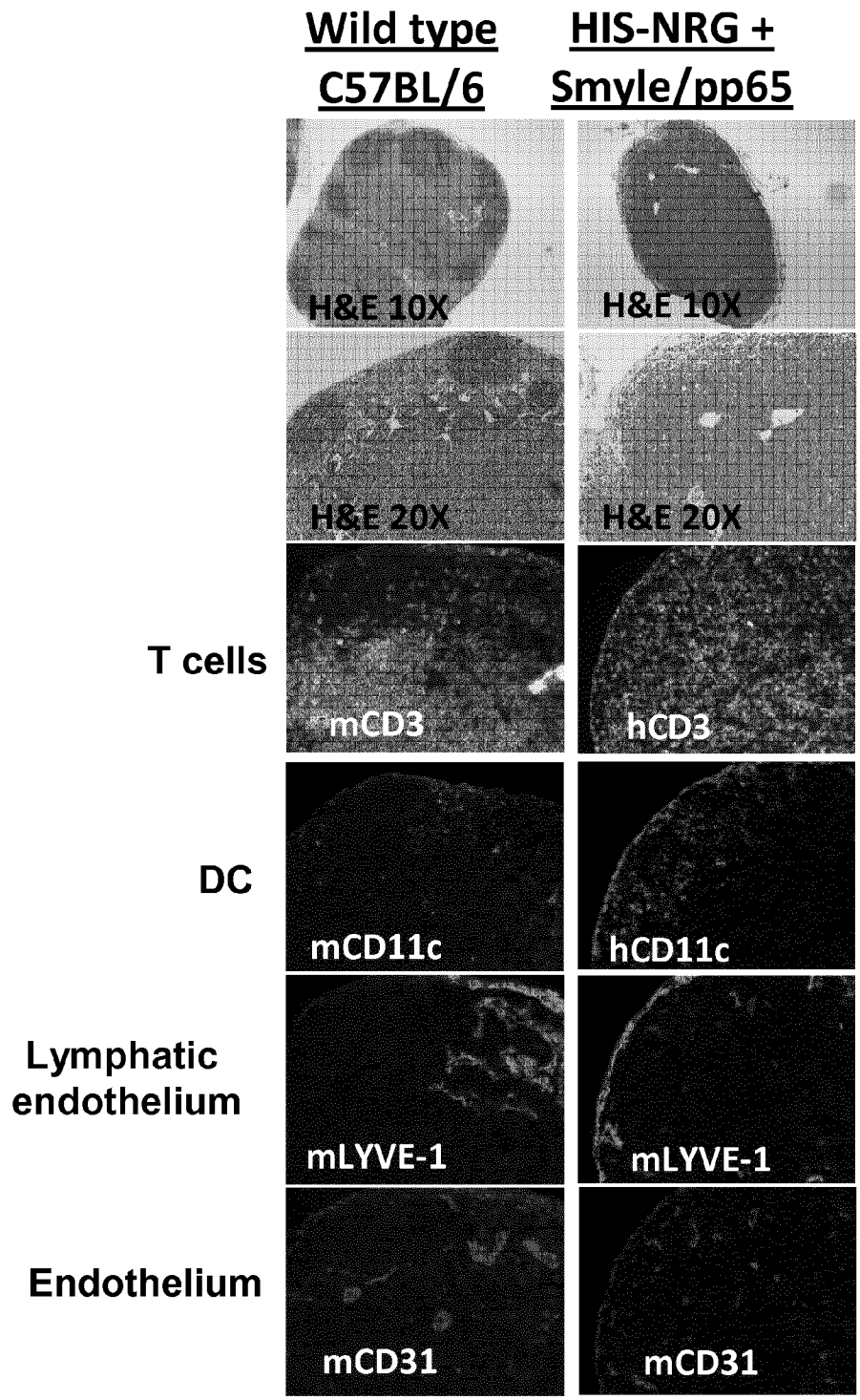
Wild type C57BL/6
HIS-NRG + Smyle/pp65
H&E 10X
H&E 10X
H&E 20X
H&E 20X
T cells
mCD3
hCD3
DC
mCD11c
hCD11c
Lymphatic endothelium
mLYVE-1
mLYVE-1
Endothelium
mCD31
mCD31

Figure 6

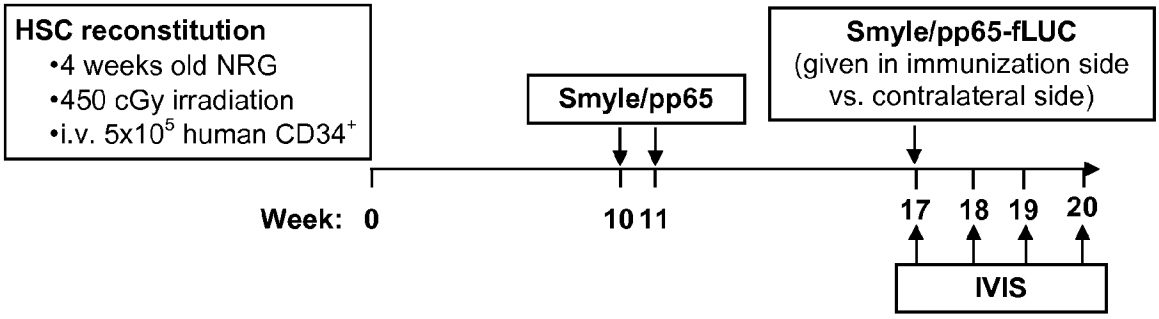

Figure 7A

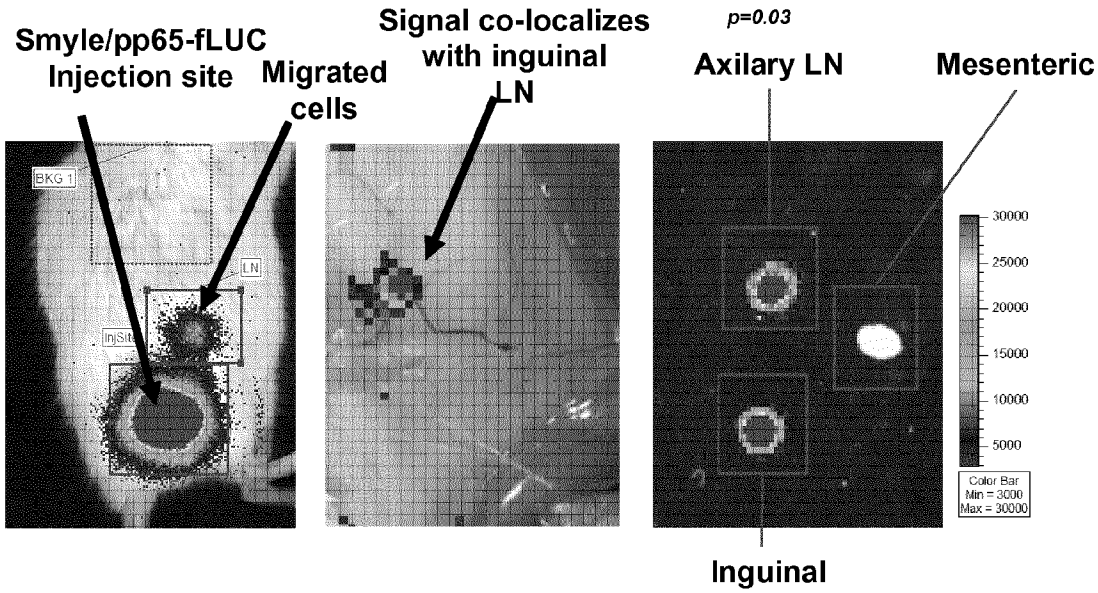

Figure 7B

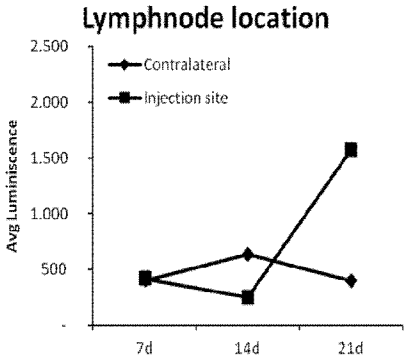

Figure 7C

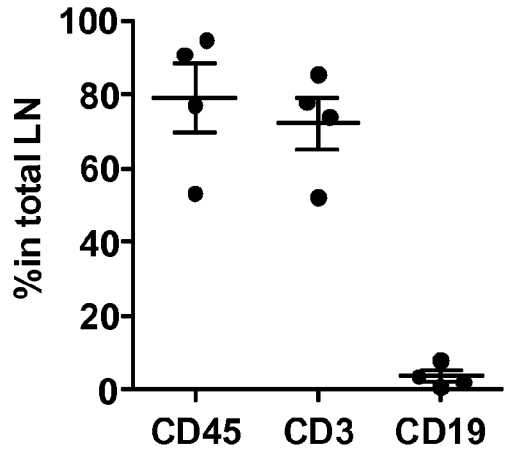

Figure 8A

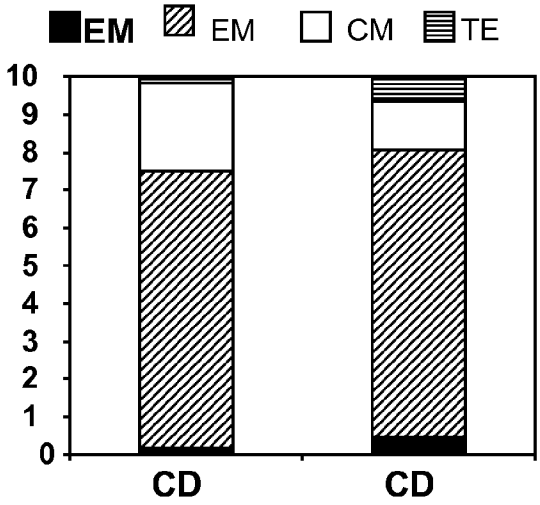

Figure 8B

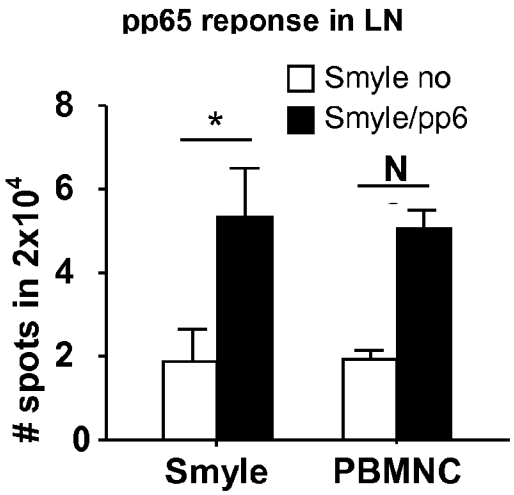

Figure 8C

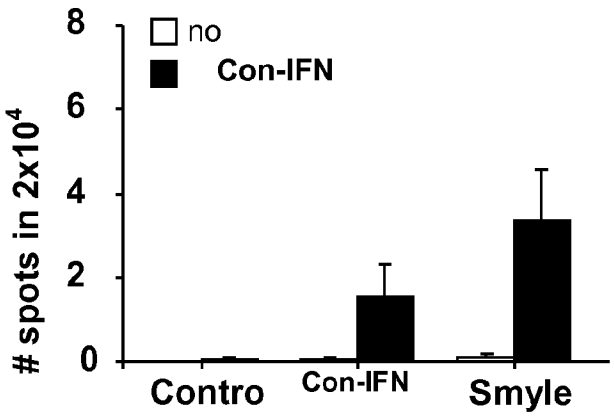

Figure 8D

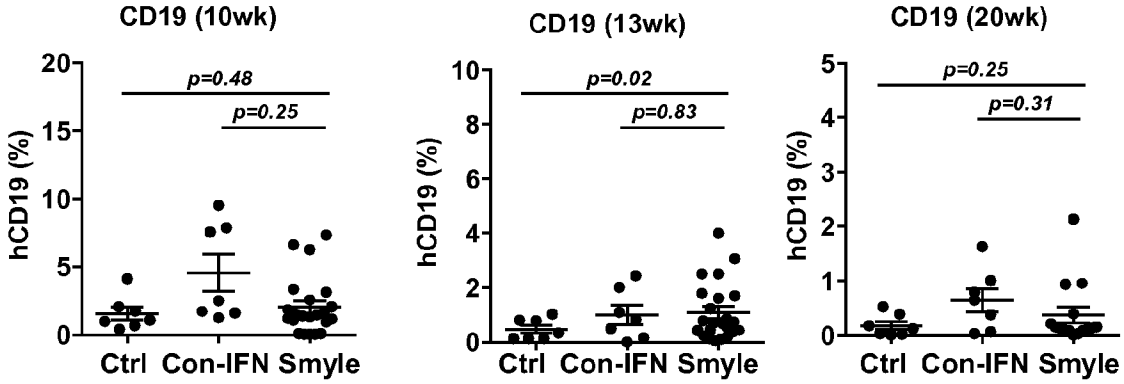

Figure 9A

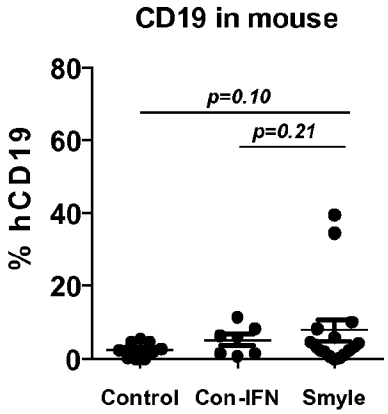

Figure 9B

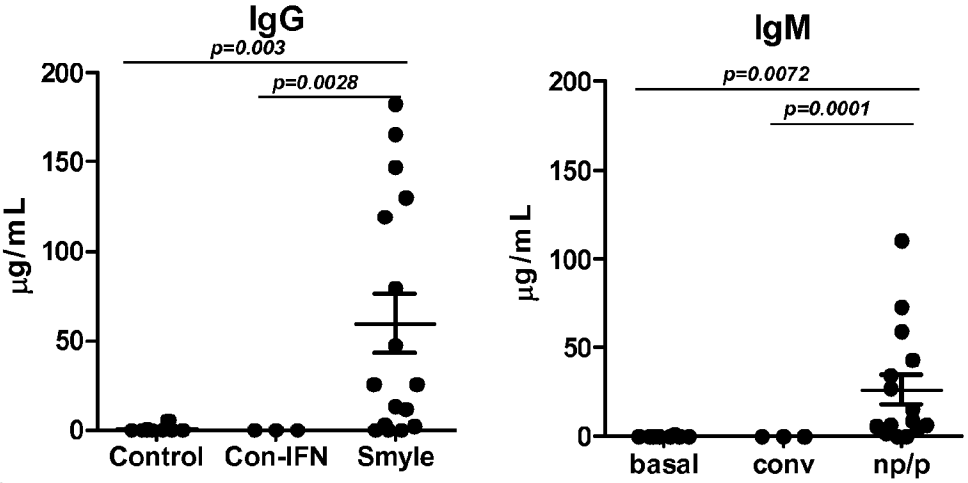

Figure 9C

INTERNATIONAL SEARCH REPORT

International application No.
PCT/EP2013/052485

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application and necessary to the claimed invention, the international search was carried out on the basis of:

   a. (means)

   ☐ on paper

   ☒ in electronic form

   b. (time)

   ☒ in the international application as filed

   ☐ together with the international application in electronic form

   ☐ subsequently to this Authority for the purpose of search

2. ☐ In addition, in the case that more than one version or copy of a sequence listing and/or table relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2013/052485

A. CLASSIFICATION OF SUBJECT MATTER
INV. A61K39/395 C12N5/0784
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
A61K C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal, BIOSIS, WPI Data, EMBASE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	BALA SAI SUNDARASETTY ET AL: "Lentivirus-Induced Dendritic Cells for Immunization Against High-Risk WT1 + Acute Myeloid Leukemia", HUMAN GENE THERAPY, vol. 24, no. 2, 10 January 2013 (2013-01-10), pages 220-237, XP055090823, ISSN: 1043-0342, DOI: 10.1089/hum.2012.128	1,13,14, 21,22
Y	the whole document	2-12, 15-20

-/--

[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
20 December 2013	08/01/2014

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Donath, Cornelia

4

Form PCT/ISA/210 (second sheet) (April 2005)

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	ANUSARA DAENTHANASANMAK ET AL: "Integrase-defective lentiviral vectors encoding cytokines induce differentiation of human dendritic cells and stimulate multivalent immune responsesand", VACCINE, ELSEVIER LTD, GB, vol. 30, no. 34, 24 May 2012 (2012-05-24), pages 5118-5131, XP028401704, ISSN: 0264-410X, DOI: 10.1016/J.VACCINE.2012.05.063 [retrieved on 2012-06-01]	1,13,14, 21,22
Y	the whole document	2-12, 15-20

X	MUDITA PINCHA ET AL: "Identity, Potency, In Vivo Viability, and Scaling Up Production of Lentiviral Vector-Induced Dendritic Cells for Melanoma Immunotherapy", HUMAN GENE THERAPY METHODS, vol. 23, no. 1, 21 December 2011 (2011-12-21), pages 38-55, XP055090824, ISSN: 1946-6536, DOI: 10.1089/hgtb.2011.170	1,13,14, 21,22
Y	the whole document	2-12, 15-20

Y	GUSTAVO SALGUERO ET AL: "Preconditioning Therapy with Lentiviral Vector-Programmed Dendritic Cells Accelerates the Homeostatic Expansion of Antigen-Reactive Human T Cells in NOD.Rag1 -/- .IL-2r[gamma]c -/- mice", HUMAN GENE THERAPY, vol. 22, no. 10, 16 May 2011 (2011-05-16), pages 1209-1224, XP055090822, ISSN: 1043-0342, DOI: 10.1089/hum.2010.215 the whole document	1-22

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