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Human innate responses and adjuvant activity of TLR ligands in vivo in mice reconstituted with a human immune system

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ABSTRACT

TLR ligands (TLR-Ls) represent a class of novel vaccine adjuvants. However, their immunologic effects in humans remain poorly defined in vivo. Using a humanized mouse model with a functional human immune system, we investigated how different TLR-Ls stimulated human innate immune response in vivo and their applications as vaccine adjuvants for enhancing human cellular immune response. We found that splenocytes from humanized mice showed identical responses to various TLR-Ls as human PBMCs in vitro. To our surprise, various TLR-Ls stimulated human cytokines and chemokines differently in vivo compared to that in vitro. For example, CpG-A was most efficient to induce IFN-α production in vitro. In contrast, CpG-B, R848 and Poly I:C stimulated much more IFN-α than CpG-A in vivo. Importantly, the human innate immune response to specific TLR-Ls in humanized mice was different from that reported in C57BL/6 mice, but similar to that reported in nonhuman primates. Furthermore, we found that different TLR-Ls distinctively activated and mobilized human plasmacytoid dendritic cells (pDCs), myeloid DCs (mDCs) and monocytes in different organs. Finally, we showed that, as adjuvants, CpG-B, R848 and Poly I:C can all enhance antigen specific $CD4^+$ T cell response, while only R848 and Poly I:C induced $CD8^+$ cytotoxic T cells response to a CD40-targeting HIV vaccine in humanized mice, correlated with their ability to activate human mDCs but not pDCs. We conclude that humanized mice serve as a highly relevant model to evaluate and rank the human immunologic effects of novel adjuvants in vivo prior to testing in humans.

1. Introduction

The most effective vaccines are live attenuated vaccines such as the yellow fever vaccine YF-17D [1] and smallpox vaccine [2,3], providing long-lasting protective immunity with a single administration. It has become clear that, by activating pathogen-encoded pattern recognition receptors (PRRs) on immune effector cells, these vaccines can efficiently activate the innate immune system to induce efficient antigen-specific humoral and cytotoxic T lymphocytes (CTL) responses [4,5]. Conversely, recombinant antigen-based vaccines are often poorly immunogenic and need co-administered adjuvants to enhance the protective immunity especially the CTL response [5].

Toll like receptor (TLRs) represent an important type of PRRs that can sense the microbial components named pathogen-associated molecular patterns (PAMPs) [6,7]. TLRs are expressed by various cells, especially by the innate immune cells such as monocytes, myeloid dendritic cells (mDCs) and plasmacytoid dendritic cells (pDCs) [6,7]. Stimulation of these innate immune cells with natural or synthetic TLR ligands (TLR-Ls) results in up-regulation of co-stimulatory molecules, enhanced expression of MHC class II molecules, and production of inflammatory cytokines [8,9]. Thus, natural ligands or synthetic agonists for TLRs are being developed as potential new vaccine adjuvants [10,11]. For example, Monophosphoryl Lipid A (MPLA), a derivative lipopolysaccharide which acts through TLR4, has been approved

Abbreviations: LN, lymph node; mDC, myeloid dendritic cell; MPLA, monophosphoryl lipid A; pDC, plasmacytoid dendritic cell; TLR-Ls, Toll-like receptor ligands.

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for clinic application as a component of AS04 adjuvant in Cervarix vaccine against cervical cancer and the vaccine against hepatitis B virus [12].

Although the adjuvant effects of TLR-Ls are promising, their immunological effects in vivo in humans are still poorly understood. Mouse models serve as the most widely used tools for mechanistic study and preclinical evaluation of TLR-Ls adjuvants. However, fundamental differences exist between human and mouse since the two species diverged between 65 and 75 million years ago, and knowledge gained from mouse studies does not always apply to humans [13,14]. For example, preclinical toxicology study in mice did not provide any indication that fialuridine would be hepatotoxic to human beings [15], but 5 of 15 clinical trial participants died and the other two required a liver transplant after receiving a nucleoside analogue fialuridine treatment due to acute liver failure. The species-specific expression of a mitochondria nucleoside transporter in human but not in mouse is probably responsible for the human-specific liver toxicity caused by fialuridine [16]. Recently, it is reported that fialuridine induced acute liver damage in human-mouse liver chimeric TK-NOG mice [17].

The immune system of human also has diverse differences from mouse [13]. One obvious difference is that bronchus-associated lymphoid tissue is only developed in mice but not in healthy humans. This has possibly evolved because mice live so much closer to the ground where they experience a higher dose of pathogens [13,18]. It has also been reported that the distribution of several TLRs in innate immune cells is quite different between human and mouse [13]. TLR9 in mouse is widely expressed on pDC, mDC, B cells and also expressed in monocyte/macrophage lineage cells [19,20], whereas in humans, it is preferentially expressed on pDCs and B cells [21,22]. TLR8, which is expressed on mDC and macrophage, can respond to ssRNA stimulation in human but this is not functional in mice [23]. Moreover, TLR10, whose ligands are as yet unknown, is widely expressed in humans but not in mice [24]. The discrepancies in TLRs distribution between mouse and human immune cells may limit the translation of findings into human clinical applications, when based on mouse work.

Another commonly used tool for evaluating the adjuvant effects of the TLR-Ls is the human peripheral blood mononuclear cells (PBMCs) in vitro culture system. However, this cell culture system is not useful to study non-circulating cells that also respond to TLR-Ls stimulation in vivo [25]. The other fundamental limitation of the human PBMCs in vitro culture system is that it cannot authentically reflect the cell-cell interaction environment in vivo. The dynamics and accessibility of the drugs to the cells should also be different in vivo compared to that in vitro. It is also difficult to evaluate vaccine adjuvant activity in inducing human T and B cell responses in vitro.

Mice reconstituted with a functional human immune system provide a valuable platform to study the development and functions of human immune cells, and more importantly, to investigate human immune response to pathogens, vaccines and other stimulations in vivo [14]. We and others have shown that injection of human $CD34^+$ hematopoietic stem cells into the immunodefecient BALB/c $Rag2^{-/-}\gamma_c^{-/-}$ mice or NOD-scid $\gamma_c^{-/-}$ (NSG) mice as well as NOD-$Rag2^{-/-}$ $\gamma_c^{-/-}$ (NRG) mice can reconstitute all major human myeloid and lymphoid subsets, including monocytes, mDCs, pDCs, T cells and B cells [26–31]. In this study, we used the humanized NRG mice as an in vivo model to explore how the human immune system responds to different PAMPs, specifically, how various TLR-Ls differentially stimulate human innate immune response and regulate adaptive $CD4^+$ helper T cell and importantly cytotoxic T lymphocyte (CTL) response to CD40-targeting HIV candidate therapeutic vaccine in vivo. We demonstrate that human leukocytes developed in humanized mice respond similarly to TLR-Ls stimulation of human PBMCs in vitro. When tested in vivo, however, TLR-Ls induce a significantly different profile of human cytokines and chemokines compared to that induced in vitro. We show that, in humanized mice, various TLR-Ls differentially activate distinct human immune cells in different lymphoid organs. Importantly, humanized mice respond to TLR-Ls stimulation differently from C57BL/6 mice [25] but similarly to that observed in nonhuman primates [32]. Finally, we show that, consistent with their different abilities to activate mDCs, Poly I:C and R848 (but not CpG-B) were able to enhance antigen-specific CTL responses to a CD40-targeting HIV candidate vaccine in humanized mice. Our study indicates that various TLR-Ls differentially activate human innate immune cells to enhance antigen-specific cellular immune responses in humanized mice. The humanized mouse model thus provides a unique platform to evaluate the immunologic effects of novel adjuvants in vivo, prior to human testing.

2. Materials and methods

2.1. Ethics statement

The report followed NIH research ethics guidelines. For the construction of humanized mouse, human fetal liver was obtained from elective or medically indicated termination of pregnancy through a non-profit intermediary working with outpatient clinics (Advanced Bioscience Resources, Alameda, CA). The use of the tissue in the research had no influence on the decision regarding termination of the pregnancy. Informed consent of the maternal donor is obtained in all cases, under regulation governing the clinic. We were provided with no information regarding the identity of the patients, nor is this information traceable. The project was reviewed by the University's Office of Human Research Ethics, which has determined that this submission does not constitute human subjects research as defined under federal regulations [45 CFR 46.102 (d or f) and 21 CFR 56.102(c)(e)(l)] and does not require IRB approval. The University of North Carolina at Chapel Hill Institutional Animal Care and Use Committee (IACUC) has reviewed and approved this research. All animal experiments were conducted following NIH guidelines for housing and care of laboratory animals and in accordance with The University of North Carolina at Chapel Hill with protocols approved by the institution's Institutional Animal Care and Use Committee (IACUC ID: 14-100).

2.2. Construction of humanized mice

We constructed humanized NRG (NOD-$Rag2^{-/-}$ $\gamma_c^{-/-}$) mice by reconstitution with human fetal liver (17 to 22 weeks of gestational age) derived $CD34^+$ hematopoietic progenitor cells (Advanced Bioscience Resources, Alameda, CA) similarly as previously reported [30]. Humanized BLT mice were generated according to a previous report [33]. Briefly, 6 to 8 weeks old NRG mice were sub-lethally irradiated and anesthetized the same day, and ~1-mm^3 fragments of human fetal thymus were implanted under the recipient kidney capsule. $CD34^+$ hematopoietic progenitor cells purified from fetal liver of same donor were injected *retro*-orbital within 3 h. Human immune cell engraftment was detected by flow cytometry 12 weeks after transplantation. All animal studies were approved by the University of North Carolina Institutional Animal Care and Use Committee (IACUC).

2.3. TLR-L treatment in vitro and in vivo

CpG-A (ODN 2216), CpG-B (ODN 2006), CpG-C (ODN 2395), R848, MPLA and Poly I:C used in this study were all purchased from InvivoGen. 1×10^6 total human PBMCs or splenocytes of humanized mice containing 1×10^6 human $CD45^+$ cells were used

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