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# The *in vitro* MIMIC® platform reflects age-associated changes in immunological responses after influenza vaccination

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ABSTRACT

Increasing research and development costs coupled with growing concerns over healthcare expenditures necessitate the generation of pre-clinical testing models better able to predict the efficacy of vaccines, drugs and biologics. An ideal system for evaluating vaccine immunogenicity will not only be reliable but also physiologically relevant, able to be influenced by immunomodulatory characteristics such as age or previous exposure to pathogens. We have previously described a fully autologous human cell-based MIMIC® (Modular IMmune *In vitro* Construct) platform which enables the evaluation of innate and adaptive immunity *in vitro*, including naïve and recall responses. Here, we establish the ability of this module to display reduced antibody production and T cell activation upon *in vitro* influenza vaccination of cells from elderly adults. In the MIMIC® system, we observe a 2.7–4.2-fold reduction in strain-specific IgG production to seasonal trivalent influenza vaccine (TIV) in the elderly when compared to adults, as well as an age-dependent decline in the generation of functional antibodies. A parallel decline in IgG production with increasing age was detected via short-term *ex vivo* stimulation of B cells after *in vivo* TIV vaccination in the same cohort. Using MIMIC®, we also detect a reduction in the number but not proportion of TIV-specific multifunctional $CD154^{+}IFN\gamma^{+}IL\text{-}2^{+}TNF\alpha^{+}$ $CD4^{+}$ T cells in elderly adults. Inefficient induction of multifunctional helper T cells with TIV stimulation in MIMIC® despite a normalized number of initial $CD4^{+}$ T cells suggests a possible mechanism for an impaired anti-TIV IgG response in elderly adults. The ability of the MIMIC® system to recapitulate differential age-associated responses *in vitro* provides a dynamic platform for the testing of vaccine candidates and vaccine enhancement strategies in a fully human model including the ability to interrogate specific populations, such as elderly adults.



## 1. Introduction

It has been estimated that it takes more than 10 years to bring a novel vaccine to market, at a cost of at least $200–500M [1]. As the majority of this cost is incurred late in the development pipeline (due to large, complex clinical trials), it is imperative to correctly identify promising candidates as early as possible. Current pre-clinical testing heavily relies on animal models, including mice and non-human primates. Besides the high cost and ethical concerns, several well-publicized clinical trial failures have revealed short-comings in the predictive validity of these animal models [2,3]. Recent technological advances in 2D and 3D culturing of human cells have led to major breakthroughs in the field of *in vitro* disease modeling. These systems range from basic immunogenicity testing via *ex vivo* culture of PBMCs to 3D human artificial lymph node (HuALN) bioreactors (reviewed in [4]). To further advance the relevance and therefore value of *in vitro* pre-clinical testing, we have developed a MIMIC® (Modular IMmune *In vitro* Construct) model solely comprised of autologous human immune cells capable of quickly and reproducibly generating antigen-specific innate and adaptive responses upon challenge. Previous work by our group has demonstrated the ability of the MIMIC® system to recapitulate *in vivo* immune profiles against

*Abbreviations:* MIMIC®, Modular IMmune *In vitro* Construct; TIV, trivalent influenza vaccine; PBMC, peripheral blood mononuclear cells; APC, antigen presenting cell; SA-HAI, surface assisted-hemagglutination inhibition assay; ICCS, intracellular cytokine staining; CDDC, cytokine-derived dendritic cell; MFI, mean fluorescence intensity; HA, hemagglutinin.

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such diverse targets as HBV, tetanus toxoid, monoclonal antibodies and YF-VAX [5–7]. In this study, we have extended those findings to include the differential immune responses of adult versus elderly donors to vaccination against seasonal influenza.

Suboptimal protective vaccine efficacy in elderly adults has been reported against pneumococcal infections, hepatitis B and influenza vaccines, with documented incidences of reduced seroconversion, seroprotection and T cell responses [8–12]. This phenomenon is often attributed to immunosenescence, the age-dependent deterioration of the immune system. Substantiating this hypothesis, a recent study exploring universal signatures of immunity post-influenza vaccination found that antibody responses correlated with age but not race, gender or diabetic status [13]. Consistent with this finding, a meta-analysis of 31 influenza vaccine studies concluded that antibody responses to trivalent influenza vaccine (TIV) were 2–4 fold lower in elderly recipients than in younger adults [10]. This reduction in serological responsiveness has practical implications, as despite higher influenza vaccination rates amongst elderly persons (~70% for elderly adults versus 38% for 18–64 year olds in the 2014–15 season) [14], influenza-associated deaths among the elderly account for almost 90% of the annual total [15]. The most useful *in vitro* systems for pre-clinical evaluation of vaccines need to not only reliably predict *in vivo* innate and adaptive immune responses but also be sensitive enough to reflect immunomodulatory characteristics intrinsic to the donor such as previous exposure history and age.

To further assess the biological relevance of the MIMIC® system, we compared the adaptive immune responses upon TIV challenge in MIMIC® with *in vivo* immune responses in adult and elderly cohorts. We demonstrated an age-dependent decline in both total binding and functional anti-TIV IgG antibody responses in the MIMIC® system that paralleled the *in vivo* antibody response profiles in the same cohort of subjects. Consistent with published studies assessing vaccination responses in elderly adults generated *in vivo* [8,16], we also observed impaired induction of antigen-specific CD4+ T cells when the responses were generated *in vitro* via the MIMIC® system. The ability of the MIMIC® system to be influenced by donor immunophysiology substantiates the value of the model and validates its relevance for pre-clinical testing. Further dissection of the MIMIC® immune response would provide unique opportunities to examine factors which contribute to immunosenescence, ultimately resulting in improved treatments for our aging population.

## 2. Materials and methods

### 2.1. Donors

Sera and PBMCs from 73 healthy donors enrolled in a Sanofi Pasteur-VaxDesign campus apheresis program (Protocol CRRI 0906009) were collected pre- and ~21 days post-influenza vaccination (Fluzone, Sanofi Pasteur). Informed consent was obtained from each subject prior to enrollment. After processing, the PBMCs were cryopreserved and stored in vapor phase liquid nitrogen until needed. Subject demographics are detailed in Table 1.

**Table 1**
Subject demographics and baseline characteristics.

| Characteristic | Young adult ($n$ = 36) | Elderly adult ($n$ = 37) |
|---|---|---|
| **Age (years)** | | |
| Mean/median | 35/36 | 69/68 |
| SD | 11 | 5 |
| Min-Max | 21–55 | 60–82 |
| **Gender, n (%)** | | |
| Male | 17 (47.2%) | 25 (67.6%) |
| Female | 19 (52.8%) | 12 (32.4%) |
| **Vaccine history** | | |
| Declared TIV within prior 4 years (%) | 10 (27.8%) | 18 (48.6%) |

### 2.2. MIMIC® studies

A MIMIC® transwell peripheral tissue equivalent module was used to generate antigen presenting cells (APCs) as previously described [17]. Briefly, pre-vaccination donor PBMCs were applied to transwells containing confluent endothelial cells (EA.hy 926, ATCC) in a 24-well plate pre-seeded with human skeletal myoblasts, followed by stimulation for 24 h with Fluzone TIV (5 ng/ml) or nothing. This dose was determined to be the optimal concentration of TIV for eliciting a detectable immune response via dose titration studies (data not shown). Differentiated APCs were harvested from the lower transwell chamber 48 h after PBMC application. Autologous $CD4^+$ T and $CD19^+$ B cells were isolated from PBMCs using magnetic enrichment kits (Stemcell Technologies), after which the B cells were cultured with TIV (5 ng/ml) or left untreated. APCs were co-cultured with autologous $CD4^+$ T cells and the pre-treated $CD19^+$ B cells for 14 days. Culture supernatants were collected for detection of total and functional anti-hemagglutinin (HA) IgG antibodies to H1, H3 or B strains via Antibody Forensics and SA-HAI, respectively.

### 2.3. $CD4^+$ T cell intracellular cytokine staining and FACS analysis

Cells from day 14 MIMIC® co-cultures were re-stimulated by autologous cytokine-derived dendritic cells (CDDCs) to determine intracellular cytokine production by $CD4^+$ T cells as previously described [18]. Briefly, one day before harvest CDDCs were pulsed with 0.36 μg/mL TIV or 1 μg/mL H3-HA or H1-HA and used 24 h later to re-stimulate T cells for 5 h in the presence of Brefeldin A (1 μg/mL). Cells were stained with LIVE/DEAD® Fixable Aqua, fixed in 2% paraformaldehyde, permeabilized with Cytofix/Cytoperm (BD Biosciences), and stained with the following panel of antibodies diluted in Perm/Wash buffer: CD4, CD154, IFNγ, IL-4, IL-5, IL-10, IL-21, TNFα (BD Biosciences), IL-2 (BioLegend) and IL-13 (eBioscience). Cells were acquired on an LSRII flow cytometer (BD Biosciences), and data were analyzed using FlowJo software v10 (FlowJo, LLC).

### 2.4. Ex vivo B cell analysis

B cells from the same donors used above were isolated from pre- and 21 days post-*in vivo* vaccination PBMCs using magnetic separation. Cells were then left unstimulated or stimulated with a mitogen cocktail (CD40L, anti-IgG/IgM/IgA antibody, IL-2, IL-10, and IL-21), and cultured for 6 days at 37 °C. Supernatants were collected for detection of total anti-HA IgG antibodies to H1, H3 or B strains via Antibody Forensics.

### 2.5. Antibody Forensics

Culture supernatants from *ex vivo* and MIMIC® studies were collected for detection of strain-specific total anti-HA IgG by a multiplexed, bead-based Antibody Forensics approach, as previously described [19]. Briefly, fluorescent carboxylated magnetic beads (Luminex) were activated and coated with 2 μg of the following recombinant HAs from Protein Sciences, Inc: A/California/07/2009 H1N1, A/Texas/50/2012, and B/Massachusetts/2/2012. This reaction was quenched after 24 h, and beads were mixed at equal volume and stored at −80 °C. The specificity of the beads was assessed by using commercially available ferret anti-sera against each of the

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