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Comparison of media for a human peripheral blood mononuclear cell-based *in vitro* vaccine evaluation system

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Purpose: Human peripheral blood mononuclear cell (PBMC)-based *in vitro* systems can be of great value in the development and assessment of vaccines but require the right medium for optimal performance of the different cell types present. Here, we compare three commonly used media for their capacity to support innate and adaptive immune responses evoked in PBMCs by Toll-like receptor (TLR) ligands and whole inactivated virus (WIV) influenza vaccine.
Materials and Methods: Human PBMCs were cultured for different periods of time in Roswell Park Memorial Institute (RPMI), Dulbecco's minimal essential medium (DMEM), or Iscove's modified DMEM (IMDM) supplemented with 10% fetal calf serum. The viability of the cells was monitored and their responses to TLR ligands and WIV were assessed.
Results: With increasing days of incubation, the viability of PBMCs cultured in RPMI or IMDM was slightly higher than that of cells cultured in DMEM. Upon exposure of the PBMCs to TLR ligands and WIV, RPMI was superior to the other two media in terms of supporting the expression of genes related to innate immunity, such as the TLR adaptor protein gene MyD88 (myeloid differentiation factor 88), the interferon (IFN)-stimulated genes MxA (myxovirus resistance protein 1) and ISG56 (interferon-stimulated gene 56), and the leukocyte recruitment chemokine gene MCP1 (monocyte chemoattractant protein-1). RPMI also performed best with regard to the activation of antigen-presenting cells. As for adaptive immunity, when stimulated with WIV, PBMCs cultured in RPMI or IMDM contained higher numbers of IFNγ-producing T cells and secreted more immunoglobulin G than PBMCs cultured in DMEM.
Conclusion: Taken together, among the different media assessed, RPMI was identified as the optimal medium for a human PBMC-based *in vitro* vaccine evaluation system.

Keywords: Peripheral blood mononuclear cells, Culture media, Influenza vaccines, Toll-like receptor, Ligands, *In vitro*

Introduction

As impressively demonstrated during the coronavirus disease 2019 (COVID-19) global pandemic over the past almost 3 years, infectious diseases can cause worldwide social, economic, financial, and healthcare crises [1]. The main evidence-based and clinical trials-proven strategy to control COVID-19 and many other infectious diseases is vaccination [2]. To improve and accelerate vaccine development, a better understanding of the basic mechanisms of immune response induction by vaccines is urgently needed. For practical and ethical reasons, in-depth characterization of these mechanisms in humans *in vivo* is difficult. In addition to this, existing animal models

also possess problems, such as high costs and poor predictive value for actual vaccine performance in humans [3].

To bridge the gap between animal experiments and clinical trials, we started some years ago to develop cell-based *in vitro* systems to compare vaccine-driven responses using human peripheral blood mononuclear cells (hPBMCs) and split and whole virus influenza and tick-borne encephalitis virus vaccines [4,5]. We found that these *in vitro* hPBMC systems are suitable for elucidating the properties of different types of vaccines and can even distinguish between vaccine batches of different quality [5].

Choosing the optimal culture medium for *in vitro* vaccine evaluation is of great importance. It is well known that the nutrient composition of the medium affects the cell metabolism, the transcriptome, and the reaction of cells to stimuli [6,7]. Different cell types can differ in their medium requirements making the choice of an optimal medium for mixed cell populations as present in hPBMCs particularly challenging. While some studies have assessed the effect of the culture medium on isolated dendritic cells, monocytes, or T cells, to our knowledge an integral investigation of medium effects on innate and adaptive immune cells in PBMCs is so far lacking [8].

Here, we applied three commonly used commercial media, Roswell Park Memorial Institute (RPMI) 1640 Medium, Dulbecco's minimal essential medium (DMEM), and Iscove's modified DMEM (IMDM), to select the one that optimally supports innate immune responses on the one hand and adaptive immune responses on the other hand in stimulated hPBMCs. RPMI is the standard medium for the isolation and culture of PBMCs. DMEM is considered as a richer medium than RPMI and is used for multiple purposes. IMDM is a derivative of DMEM which is frequently used for T cell assays [9]. The three media differ in many aspects (Supplement 1). Most importantly, RPMI contains lower levels of most amino acids, vitamins, and particularly calcium and glucose but much higher levels of phosphate than DMEM and IMDM. DMEM and IMDM are overall rather similar but differ in some vitamins, magnesium and sodium content, and the absence or presence of 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES). Using Toll-like receptor (TLR) agonists and whole inactivated virus (WIV) influenza vaccine as stimulators we investigated the effect of the media on viability, expression of innate immune response factors and activation markers, and the activation of T and B cells. Considering all parameters studied, RPMI turned out to be superior to DMEM and IMDM for use in our *in vitro* PBMC-based vaccine evaluation system.

Materials and Methods

Toll-like receptor ligands and influenza vaccine

The TLR ligands, lipopolysaccharide (LPS; standard grade, from *Escherichia coli* K12) and resiquimod (R848), were both purchased from Invivogen (Toulouse, France). H5N1 virus (NIBRG-23, a reassortant prepared by reverse genetics from A/turkey/Turkey/1/2005 [H5N1] virus and A/PR/8/34 [H1N1] virus) was obtained from the National Institute for Biological Standards and Control (Potters Bar, UK) and propagated in Madin-Darby canine kidney cells. H5N1-derived WIV was produced and purified as described previously [4].

Media

RPMI 1640, HEPES (RPMI; cat: 52400041), DMEM, high glucose, GlutaMAX supplement, pyruvate (DMEM; cat: 31966047), and IMDM, GlutaMAX supplement (IMDM; cat: 31980030) were obtained from Gibco (Life Technologies, Paisley, UK and Grand Island, NY, USA) and completed by the addition of 10% fetal bovine serum (FBS; Life Science Production, Sandy, UK), 2% penicillin/streptomycin (Life Technologies, USA), and 50 μM 2-mercaptoethanol (Life Technologies, UK).

Human primary cells

Human buffy coats were obtained from the Dutch Blood Bank (Sanquin, Nijmegen, The Netherlands). PBMCs were isolated from buffy coats using Ficoll-Paque medium (Cytiva, Uppsala, Sweden) as previously described [4], and were cryopreserved in 90% FBS and 10% dimethylsulfoxide (DMSO; Millipore, Billerica, MA, USA) mixture until use.

In vitro stimulation of hPBMCs with influenza vaccine, LPS, and R848

PBMCs were thawed in a water bath and pooled in RPMI medium, as previously described [4]. After counting with Trypan Blue, the PBMCs were divided over three tubes and the RPMI medium was removed using centrifugation. Subsequently, the PBMCs were resuspended in the corresponding medium and seeded into a 24-well plate at a density of 1×10^6 cells/mL/well. After resting the cells for 15 minutes at 37°C with 5% CO_2, the stimulants, such as WIV (10 μg/mL hemagglutinin), LPS (1 μg/mL), or R848 (1 μg/mL), were added in corresponding wells. If cells were cultured for extended periods, half of the media was replaced on day 5 with fresh completed

media without adding new stimuli.

Multiparameter flow cytometry

On day 1, 7, and 10, the cell viability was checked with LIVE/DEAD Fixable Blue Dead Cell Stain Kit (Invitrogen, Eugene, OR, USA). On day 1, the presence of the activation markers CD40, CD80, CD86, and major histocompatibility complex II (MHC-II) was assessed by flow cytometry. To this end, 0.5×10^6 cultured PBMCs were pipetted into a deep well plate and washed with wash buffer (1×Dulbecco's Phosphate Buffered Saline [Gibco] with 2% fetal calf serum) and subsequently stained. The fluorophore panel for all antibodies was selected by Cytek Biosciences as follows: LIVE/DEAD Fixable Blue Dead Cell Stain Kit (Invitrogen), BUV805-CD11C, BuV737-CD86, PE-Cy5-CD80 (BD Bioscience, San Jose, CA, USA), BV711-HLA-DR, and PE-CD40 (Biolegend, Fell, Germany). The staining was performed for 30 minutes at 4°C. After washing, the stained cells were fixed with 4% paraformaldehyde.

Flow cytometry was performed on a Cytek Aurora flow cytometer (Cytek Biosciences, Amsterdam, The Netherlands). Data were recorded and unmixing plots were extracted by Spectroflo software (Cytek Biosciences). Analysis was performed using Kaluza software (Beckman Coulter, Woerden, The Netherlands).

RNA isolation and quantitative polymerase chain reaction

On days 1, 7, and 10 after stimulation, cells were centrifuged at 300 g for 5 minutes. The supernatants were collected and stored at -20°C. Cell pellets and remaining cells in the 24-well plate were completely collected in Trizol Reagent (Invitrogen, Carlsbad, CA, USA) and the RNA was isolated according to the manufacturer's instructions. After isolation, the RNA was resuspended in diethylpyrocarbonate-treated water (Thermo Fisher Scientific, Waltham, MA, USA) and stored at -80°C.

To perform reverse transcription quantitative polymerase chain reaction (qPCR), the PrimeScript RT reagent kit with genomic DNA (gDNA) Eraser (Takara Bio, Kusatsu, Japan) was applied to thawed RNA samples to eliminate gDNA and enforce reverse transcription. Expression of myeloid differentiation factor 88 (MyD88), myxovirus resistance protein 1 (MxA), interferon-stimulated gene 56 (ISG56), monocyte chemoattractant protein-1 (MCP1) (day 1 and day 2), interleukin 2 (IL2), interferon γ (IFNγ), B-lymphocyte-induced maturation protein 1 (BLIMP1), and X-box-binding protein 1 (XBP1) (day 7 and day 10) was detected using SYBR Green dye. Primer sequences are provided in Supplement 2. The threshold cycle of samples was recorded by a CFX96 System (Bio-Rad, Hercules, CA, USA) and the gene expression levels were normalized against glyceraldehyde-3-phosphate dehydrogenase (GAPDH). Data were analyzed by the comparative cycle threshold method and are expressed as fold change [10].

Enzyme-linked immunosorbent assay of total IgG

On day 10, cell supernatants were collected and stored at -20°C, and 100 μL cell supernatant was thawed when needed and total immunoglobulin G (IgG) content was determined using the IgG (total) human uncoated enzyme-linked immunosorbent assay (ELISA) kit (Invitrogen, New York, NY, USA) according to the manufacturer's instructions. The results were measured on a GloMax Discover Microplate Reader (Promega Corp., Madison, WI, USA).

Enzyme-linked immunospot assay for IFNγ

To determine the number of IFNγ-secreting T cells, PBMCs cultured and stimulated in the different media were collected on day 10.2×10^5 cells were seeded in the wells of an activated and coated enzyme-linked immunospot assay (ELISpot plate; Mabtech, Nacka Strand, Sweden), and processed as previously described [4]. IFNγ-secreting T cells were quantified by an AID ELISpot/Fluorospot reader (Autoimmun Diagnostika GmbH, Straβberg, Germany).

Results

Cell viability

To optimize the earlier developed human cell-based vaccine evaluation system, we exposed PBMCs in RPMI, DMEM, or IMDM to the TLR ligands LPS and R848, or to influenza WIV and used various techniques to assess innate and adaptive immune responses.

We first determined the viability of human PBMCs without stimulation in the different media over time, using flow cytometry to quantify live and dead cells. We observed that on day 1, the viability of PBMCs was >80% in all media with only minor medium-related differences. Over time, viability decreased in all media. On day 7 and day 10, the decrease in viability tended to be more prominent for PBMCs cultured in DMEM than for cells cultured in RPMI or IMDM (Fig. 1). This tendency was observed for four of the five donors studied, though the differences were not statistically significant due to high variability among the donors.

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Innate immune responses

Next, to reveal the effect of different media on the induction of innate immune responses we stimulated PBMCs cultured in the different media with TLR agonists or WIV and assessed the transcription levels of MyD88, MxA, ISG56, and MCP1, four genes involved in antiviral responses. MyD88 is the canonical adaptor for most of the TLRs [11]. MxA is a reliable marker for viral-induced type I interferon bioactivity and ISG56 can be directly and strongly induced by a range of viruses including influenza virus [12]. MCP1 is relevant for various respiratory infections by fostering leukocyte recruitment [13].

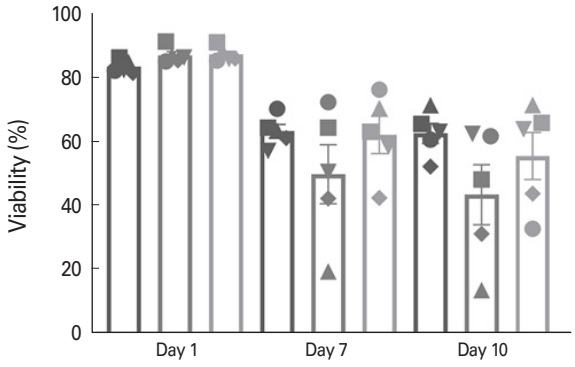

Fig. 1. Viability of peripheral blood mononuclear cells (PBMCs) cultured in different media for different time periods. PBMCs from 5 healthy donors were cultured in Roswell Park Memorial Institute (RPMI; dark grey symbols), Dulbecco's minimal essential medium (DMEM; medium grey symbols), and Iscove's modified DMEM (IMDM; light grey symbols) without further stimulation. Each symbol represents a single donor. On days 1, 7, and 10, cells were harvested and viability was assessed by LIVE/DEAD Fixable Blue staining.

Upon stimulation of PBMCs with WIV, the expression of the genes encoding ISG56 and MxA was higher in PBMCs cultured in RPMI than in PBMCs cultured in the other two media for four out of five donors and MyD88 expression was higher in RPMI and DMEM than in IMDM for three of the five donors (Fig. 2A–C). Yet, due to variations among donors, the observed differences did not reach statistical significance. No consistent differences were found for MCP1 expression (Fig. 2D).

As for samples stimulated with TLR agonists, PBMCs cultured in RPMI exhibited the highest expression of MxA and MCP1 when exposed to LPS (four out of five donors) or R848 (three out of five donors) (Fig. 2B, D). ISG56 expression induced by LPS stimulation did not show a clear association with the medium used. However, ISG expression induced by R848 did, with four out of five donors exhibiting the highest expression when cultured in DMEM (Fig. 2C). MyD88 transcription levels were very low upon stimulation with either LPS or R848 (with one exception) and did not allow any conclusion regarding medium effects (Fig. 2A).

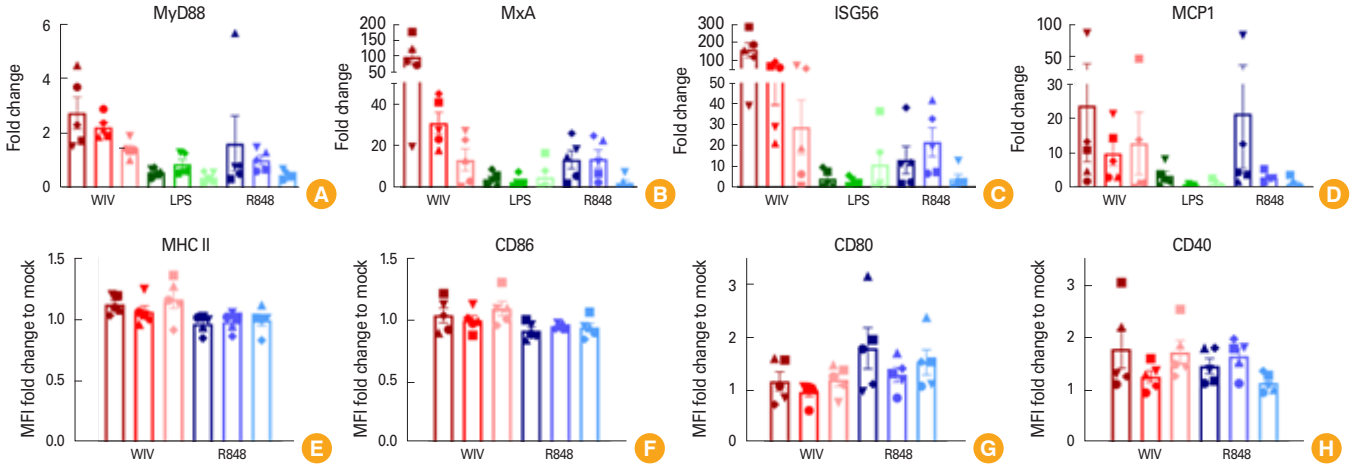

Fig. 2. Activation of innate immune responses in peripheral blood mononuclear cells (PBMCs) cultured in different media (darkest color for Roswell Park Memorial Institute [RPMI], medium color for Dulbecco's minimal essential medium [DMEM], and lightest color for Iscove's modified DMEM [IMDM]). PBMCs from five healthy donors were incubated with different stimulants (whole inactivated virus [WIV]-red, lipopolysaccharide [LPS]-green, and resiquimod [R848]–blue) and collected on day 1 **(A–D)** to measure MyD88 **(A)**, MxA **(B)**, ISG56 **(C)**, and MCP1 **(D)** expression levels with quantitative polymerase chain reaction. Bars in A–D represent the mean fold changes that were normalized to the glyceraldehyde-3-phosphate dehydrogenase (GAPDH) level and compared to the non-stimulated control by using the equation $2^{-\Delta\Delta Ct}$. Dendritic cell activation was determined by measuring the median fluorescence intensity (MFI) for major histocompatibility complex II (MHC-II) **(E)**, CD86 **(F)**, CD80 **(G)**, and CD40 **(H)** under gating for CD11c+ cells. Each symbol represents a single donor. Results are expressed as MFI fold change as compared to the corresponding mock-treated cells.

In order to assess the activation of dendritic cells, the main antigen-presenting executors in innate immunity, we measured various activation markers, such as MHC II, CD40, CD80, and CD86 using flow cytometry. MHC II molecules mainly execute antigen presentation to CD4 T cells leading to adaptive immune responses [14]. CD40 is crucial in dendritic cell activation and can trigger costimulatory molecules such as CD80 and CD86 that promote efficient T cell activation [15,16].

WIV only affected CD40 expression, though to a moderate extent. In four out of the five donor samples, this effect was stronger in RPMI and IMDM than in DMEM (Fig. 2H). R848 had no (MHC II, CD86) or moderate (CD80, CD40) effects on activation marker expression (Fig. 2E–H) without obvious medium effects except that CD40 expression was generally lower in IMDM than in the other two media (Fig. 2G, H).

Adaptive immune responses

In order to gain a more comprehensive picture of the effects of different media, we also investigated adaptive immune responses of T cells and B cells on day 7 and day 10 using qPCR, ELISA, and ELISpot.

We first determined transcription levels of IL2 and IFNγ, two cytokines known to be expressed by influenza virus-specific T cells [4]. When comparing the responses for a given donor across the different media, we found that the medium best supporting IL2 and IFN-γ transcription varied among the donors, independent of the stimulus used (Fig. 3A–D) with a tendency to higher IL2 responses in DMEM and rather inconsistent results for IFNγ responses.

Next, we determined the number of IFNγ-secreting T cells using ELISpot. It turned out that PBMCs cultured with RPMI and IMDM contained almost equal numbers of IFNγ-secreting T cells which were slightly higher than for PBMCs cultured with DMEM (Fig. 3E). This trend was visible for four out of the five donors.

To assess B cell responses, the gene expression of BLIMP1 and XBP1, transcription factors that contribute to plasma cell differentiation [17], was assessed on day 7 and day 10. BLIMP1 expression was upregulated by WIV and R848 and somewhat less by LPS and overall upregulation was higher in RPMI and DMEM than in IMDM (Fig. 4A, B). XBP1 expression levels were only affected by R848, most prominently in IMDM (day 7) followed by DMEM (day 10) (Fig. 4C, D).

Finally, we investigated total IgG levels to assess humoral immune responses. The total IgG levels of PBMC cultures stimulated in RPMI and IMDM were slightly higher than those of PBMC cultures in DMEM in two of the three donor samples that did produce antibodies upon stimulation with WIV (Fig. 4E).

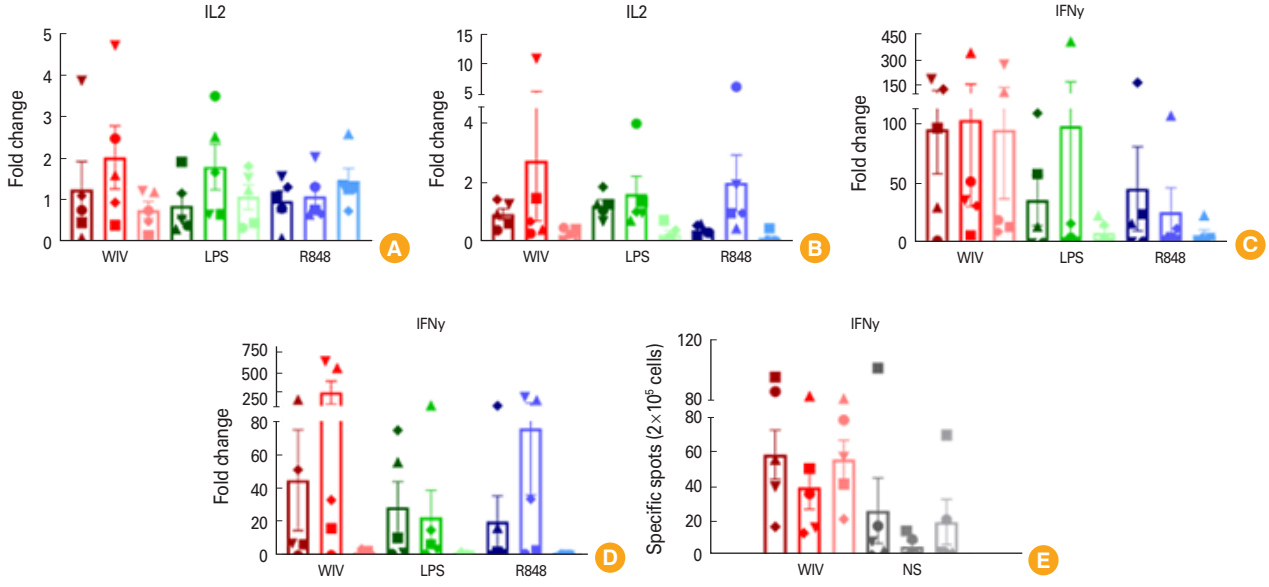

Fig. 3. T cell responses for peripheral blood mononuclear cells (PBMCs) cultured in different media. To reveal the cellular immune responses in different media, RNA of PBMCs (treated as described in the legend to Fig. 2) was isolated to quantify the interleukin 2 (IL2) **(A, B)** and interferon γ (IFNγ) **(C, D)** gene expression on day 7 **(A, C)** and day 10 **(B, D)**. On day 10, the number of IFNγ-producing T cells was assessed by enzyme-linked immunospot assay (ELISpot assay) **(E)**. Each symbol represents a single donor. WIV, whole inactivated virus; LPS, lipopolysaccharide; R848, resiquimod; NS, non-stimulated.

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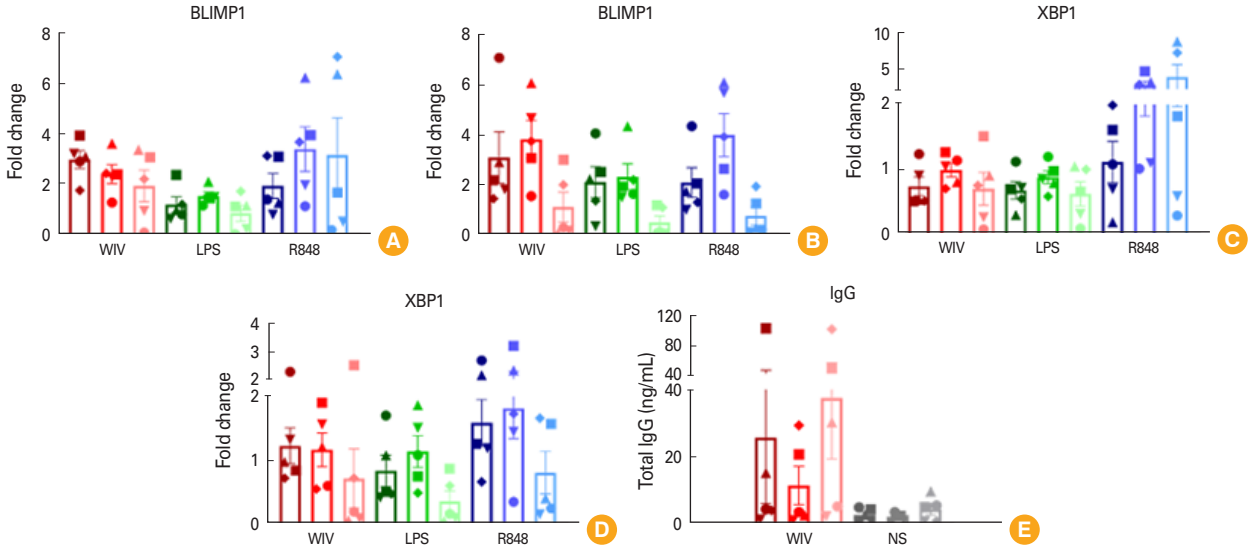

Fig. 4. B cell-related responses in peripheral blood mononuclear cells (PBMCs) cultured in different media. To get insight into the effect of the different media on B cell responses, the expression levels of B-lymphocyte-induced maturation protein 1 (BLIMP1) **(A, B)** and X-box-binding protein 1 (XBP1) **(C, D)**, were evaluated on day 7 **(A, C)** and day 10 **(B, D)** in PBMC cultures treated as described above. The total immunoglobulin G (IgG) secretion in cell supernatants was measured using enzyme-linked immunosorbent assay **(E)**. Each symbol represents a single donor. WIV, whole inactivated virus; LPS, lipopolysaccharide; R848, resiquimod; NS, non-stimulated.

Discussion

This study was dedicated to fine-tuning a human PBMC-based system for *in vitro* vaccine evaluation by selecting the optimal medium from three commonly used media, RPMI, DMEM, and IMDM. Collectively, compared to cells cultured in DMEM and IMDM, PBMCs cultured in RPMI had the highest viability in long-term culture. After being stimulated with WIV, PBMCs cultured with RPMI also possessed the highest transcription levels of innate immunity-related genes and—together with PBMCs cultured in IMDM-displayed the highest numbers of IFNγ-secreting T cells and the highest levels of total IgG. Thus, across all parameters studied, RPMI was the best of the three media evaluated for our human PBMC-based vaccine evaluation *in vitro* system.

We found very few studies which assessed the effect of different media on hPBMCs. Liu et al. [18] compared the mitogen-induced proliferation of hPBMCs in RPMI, DMEM, and IMDM and found the highest proliferation rate in DMEM. Yet, nor the expression of innate immunity-related genes and surface markers nor the activation of T cells was measured, precluding further comparison with our data. Chen et al. [19] investigated the effect of RPMI and IMDM on human monocyte-derived dendritic cells. CD14 and CD83 expression as well as the endocytic capacity were similar in the two media. However, RPMI-cultured dendritic cells more effectively stimulated T cell proliferation which is in line with our results. However, contradictory results were found by Xu et al. [9] who observed that IMDM was superior to RPMI in supporting T cell proliferation. This study used CD3/CD28 T cell stimulation [9]. Other responses of the cells, e.g., cytokine production, were not studied. Thus, to our knowledge, this is the first study which assesses in an integral way medium effects on the activation of myeloid as well as lymphoid cells in hPBMC cultures.

Interestingly, in our study, the two nutrient-rich media, DMEM and IMDM, did not lead to higher cell survival in our human PBMC-based *in vitro* system nor to better performance regarding vaccine-stimulated immune responses. In fact, at both day 7 and day 10, the cell viability was poorest and highly variable for cells cultured in DMEM while the numbers of IFNγ-secreting T cells and the total IgG amount were rather low. This result suggests that the outcomes of adaptive immunity might be determined largely by cell viability.

Is there a difference in composition between RPMI and IMDM on the one hand and DMEM on the other hand which may explain the poor performance of the latter? According to the formulation of those three media (Supplement 1), both RPMI and IMDM contain 20 different amino acids, but DMEM contains only 15 amino acids. Amino acids are vital building blocks for proteins and deprivation of amino acids may lead to cell death [20]. In terms of vitamin species, DMEM

contains three fewer species than RPMI and two fewer species than IMDM. Vitamins have a positive influence on cell proliferation [21]. For example, biotin is required for fatty acid synthesis and prolongs cell life span by increasing ATP production [22]. Vitamin B can have a significant and direct effect on cell proliferation and apoptosis by affecting cell metabolism [23]. In addition to the nutrients, we believe that also a stable pH is a key to cell viability [24]. RPMI and IMDM both contain 25.03 mM HEPES, while DMEM is buffered with bicarbonate only. Yao et al. [25] reviewed that when HEPES is present in a medium, the pH is more stable than with only bicarbonate. We suggest that the reduced viability of PBMCs cultured in DMEM on day 7 and day 10 could be attributed to the incomplete amino acids, and vitamins and the absence of HEPES to maintain a stable pH value. Monitoring of the pH value over the time of the culture is therefore recommendable.

In comparison with RPMI-cultured PBMCs, IMDM-cultured cells slightly underperformed in cell viability under long-term culture and exhibited particularly low transcription of immune-related genes. This result was unexpected because IMDM is neither deficient in amino acids nor in vitamins compared to RPMI. Besides, IMDM also contains very high concentrations of nutrients, such as glucose and glutamine. Generally, in a medium, glucose is a vital energy resource [26], and glutamine is very important as a carrier of nitrogen and carbon, and as a source of energy if demand is high [27]. However, our *in vitro* system was established based on human PBMCs that may not need very high amounts of nutrients. In fact, IMDM was developed to support cell proliferation rather than to mimic real physiological conditions [7,25]. For example, the concentrations of glucose and glutamine in IMDM are much higher than in human plasma. The higher glucose content may lead to higher amounts of lactic acid. Lactic acid can interfere with cellular metabolism and regulate the inflammation of PBMCs and monocytes [28]. A by-product of amino acid degradation, especially of glutamine, is ammonia, which can be a stress factor for cells. Elevated ammonia concentrations can increase the consumption of glutamine, raise the intracellular pH level and result in cell death [29]. Thus, for the elucidation of vaccine effects on different cell types in hPBMCs a less rich medium may be advantageous.

Our study has some limitations. First, we included a rather small number of donors in our study. It is well known that responses in PBMCs can vary substantially among cells from different donors, often precluding statistical significance of observed differences, even with larger numbers of donors [30]. We indeed saw substantial donor-to-donor variation. However, we compared in our study the responses of the same donor sample across different media. Overall, we observed that most donor samples showed the same preference and disfavor of media for the parameters studied. We regard this as additional support for our conclusion. Second, we performed our study with frozen rather than with fresh PBMCs. We did so because in many studies, fresh PBMC samples are not available or samples taken at different time points are to be compared. In a previous paper, we assessed the responses of fresh versus frozen/thawed PBMCs and observed that they showed the same trend regarding the upregulation of relevant marker genes although the absolute fold changes differed slightly [31]. Nonetheless, the present study focused on the comparison of different media and we consider it unlikely that a given medium supports the activation of frozen but not fresh cells and vice versa. Third, we used only WIV as a vaccine representative in our study. Indeed, the sort of trigger will determine which innate immune pathways are activated and media might differ in how well they support different immune pathways [32]. Next to WIV, we also investigated the effects of LPS and R848, triggers of TLR4 and TLR7/8, respectively, but a future study should include the assessment of responses to bacterial vaccines. Finally, we stimulated hPBMCs with WIV and performed an ELISPOT assay to detect the number of influenza-specific IFNγ-secreting T cells. Although T cells are the main secretors of IFNγ, we cannot exclude that NK cells might also have been picked up as IFNγ-producing cells.

Taken together, we systematically compared the effect of the culture medium on the activation of a range of innate and adaptive immune responses in PBMCs by a whole virus vaccine and different TLR ligands. Among the three common media we tested, RPMI was the optimal medium to support immune cell activation. Overall, we believe that an optimal medium for a human PBMC-based *in vitro* system for vaccine evaluation should be able to adequately maintain the pH and be rich in amino acid and vitamin species. At the same time, extremely high nutrient concentrations are not particularly necessary and may even be counterproductive. However, in the future, more physiological media intended to more closely resemble the metabolic composition of human blood, such as Plasmax and human plasma-like medium should be tested in comparison with RPMI [6,33-37].

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Supplementary Materials

Supplementary materials are available at Clinical and Experimental Experimental Vaccine Research website (http://www.ecevr.org).

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