Data Article

Data correlations between gender, cytomegalovirus infection and T cells, NK cells, and soluble immune mediators in elderly humans

Ahmad Al-Attar, Steven R. Presnell, Charlotte A. Peterson, D. Travis Thomas, Charles T. Lutz

Article info

Article history:
Received 8 April 2016
Received in revised form 30 May 2016
Accepted 7 June 2016
Available online 15 June 2016

Keywords:
NK cell
Ageing
Cytomegalovirus
T cell
Sex factors
Vitamin D
C-reactive protein
Adiponectin
IL-15
Sphingosine-1-phosphate
Retinol binding protein-4

Abstract

We describe a cohort of 50 elderly subjects, age at least 70 years. We present gender-specific findings in T lymphocyte markers and soluble immune mediators. We show the correlation between cytomegalovirus infection status with CD56dim NK cell responses to a variety of stimuli and with CD56bright/CD56dim NK cell ratio. We also present the correlation of retinol binding protein (RBP) – 4 plasma levels with NK cell responses and we explore the relationship between gender and adiponectin, 25(OH)D (vitamin D), and RBP4 in affecting CD56dim NK cell responses. These data are discussed in Al-Attar et al. (2016) [1].

© 2016 The Authors. Published by Elsevier Inc. This is an open access article under the CC BY license (http://creativecommons.org/licenses/by/4.0/).
**Specifications Table**

| Subject area | Immunology |
|-------------|------------|
| More specific subject area | Sex differences in immune cells and soluble mediators |
| Type of data | Tables |
| How data was acquired | Flow cytometry and enzyme-linked immunosorbent assay |
| Data format | Analyzed |
| Experimental factors | Immune cells were analyzed immediately ex vivo or were stimulated in vitro |
| Experimental features | Correlations and mean differences were calculated |
| Data source location | Lexington, KY – USA |
| Data accessibility | Data are within this article |

**Value of the data**

- Researchers need to be aware of the gender differences in NK cell responses to various stimuli.
- Exposure to cytomegalovirus (CMV) affects immune responses from T and NK cells and could therefore be an important factor to consider when performing research in elderly human subjects.
- Levels of soluble plasma immune mediators adiponectin and vitamin D (25(OH)D) which affect NK cell development and activity are higher in elderly women compared to men, and are important factors to consider when studying human NK cells.

1. **Data**

Enclosed are data concerning T cell markers and subsets found in elderly women and men (Table 1). Also shown is the effect of cytomegalovirus (CMV) infection on CD56<sup>dim</sup> NK cell responses to a variety of stimuli and on the CD56<sup>bright</sup>/CD56<sup>dim</sup> NK cell ratio in blood lymphocytes (Table 2). We present the levels of various plasma immune mediators and their levels in elderly women and men (Table 3). We show how plasma RBP4 level correlates with NK cell responses in vitro (Table 4) and we explore if the interaction between gender and plasma adiponectin, RBP4, and 25(OH)D (vitamin D) affects CD56<sup>dim</sup> NK cell responses in vitro (Table 5). Full details of the data can be found in Al-Attar et al. [1].

2. **Experimental design, materials and methods**

Male and female subjects > 70 years were recruited from volunteer donor pools at the University of Kentucky Sanders-Brown Center on Aging and by advertisements. Venous blood from 26 males (age mean ± standard error of the mean (SEM), 77.8 ± 0.31, range 70–90 years) and 24 females (age mean ± SEM, 77.0 ± 0.91, range 70–85 years) were analyzed between October 2012 and April 2014. Prospective donors were screened by telephone interview to exclude those with conditions previously demonstrated to affect NK cells. Exclusion criteria included hospitalization or serious illness in the prior year, history of immunologic illness (rheumatoid arthritis, systemic lupus, scleroderma, polymyositis, Sjögren's syndrome, transplantation, etc), current use of immunomodulatory medications (e.g., corticosteroids), inability to walk one city block, regular consumption of two or more alcoholic beverages per day (28 g ethanol), diabetes, and a history of cancer within the last 10 years, except non-melanoma skin cancers. Two female subjects (but no male subjects) received hormone replacement therapy, one with topical estrogen and one with topical estrogen, progesterone, and testosterone. The gender differences affecting CD56<sup>dim</sup> NK cell CD38 density and the CD56<sup>bright</sup> to CD56<sup>dim</sup> NK cell ratio were no longer significant when the hormone replacement subjects were excluded from analysis. All other gender differences reported below remained significant after exclusion of these two subjects. Blood samples were obtained from people without acute
| Cell Subset | Sex | Mean | SEM | P value |
|-------------|-----|------|-----|---------|
| T cell CD4/8 ratio | ♀ | 3.37 | .45 | .79 |
| | ♂ | 3.56 | .56 | |
| T cell Ki67 | ♀ | 3.59 | .22 | .59 |
| | ♂ | 3.43 | .20 | |
| T cell CD4 | ♀ | 66.67 | 2.68 | .83 |
| | ♂ | 67.50 | 2.74 | |
| CD4 CD57 | ♀ | 6.34 | 1.15 | .51 |
| | ♂ | 5.33 | .95 | |
| CD4 CD28 | ♀ | 95.36 | 1.31 | .46 |
| | ♂ | 96.55 | .96 | |
| CD4 HLA-DR | ♀ | 25.74 | 2.05 | .70 |
| | ♂ | 24.96 | 1.45 | |
| CD4 HLA-DR gMFI | ♀ | 126.98 | 23.02 | .34 |
| | ♂ | 83.87 | 5.21 | |
| CD4 CD38 | ♀ | 45.77 | 3.99 | .12 |
| | ♂ | 43.52 | 1.44 | |
| CD4 CD38 gMFI | ♀ | 175.60 | 21.15 | .30 |
| | ♂ | 138.72 | 5.52 | |
| T cell CD8 | ♀ | 25.96 | 2.36 | .73 |
| | ♂ | 27.23 | 2.66 | |
| CD8 CD57 | ♀ | 47.83 | 4.28 | .92 |
| | ♂ | 47.20 | 4.51 | |
| CD8 CD28 | ♀ | 50.55 | 5.26 | .94 |
| | ♂ | 50.02 | 5.02 | |
| CD8 HLA-DR | ♀ | 65.45 | 3.71 | .79 |
| | ♂ | 66.72 | 2.89 | |
| CD8 HLA-DR gMFI | ♀ | 294.02 | 35.63 | .68 |
| | ♂ | 275.66 | 28.01 | |
| CD8 CD38 | ♀ | 22.02 | 2.68 | .35 |
| | ♂ | 17.12 | 1.29 | |
| CD8 CD38 gMFI | ♀ | 76.29 | 6.20 | .27 |
| | ♂ | 68.71 | 3.65 | |

For each tested analyte on gated T cell subset, the mean and standard error of the mean (SEM) is shown for each gender. All values are given as percentage of the gated population, except when geometric mean fluorescence intensity (gMFI) antigen level is given. Statistical significance of mean differences was determined by student’s T test.
illness in the preceding week between 9:30 a.m. and 12:45 p.m. Lymphocytes and serum 25-hydroxyvitamin D (25(OH)D) were analyzed immediately; plasma was aliquoted and stored at −80 °C. All human subjects research was approved by the University of Kentucky Institutional Review Board.

2.1. ex vivo staining

Whole blood was diluted 1:1 with PBS and overlaid on Lymphoprep™ lymphocyte separation medium (Axis-Shield, Oslo, Norway) according to the manufacturer’s protocol. Peripheral blood mononuclear cells (PBMC) were collected and washed twice. For ex vivo staining, ~0.5 × 10⁶ PBMC were washed and incubated with human IgG for 15 min at room temperature to block Fc-receptor binding and then stained on ice for 30 min with combinations of fluorescently labeled mAb (See Supplementary Table S1 in Ref. [1]). After washing, the cells were analyzed on a LSR-II flow cytometer (BD, Franklin Lakes, NJ). CD4 and CD8 T cells were divided into four subpopulations, T N: CD62L⁺ CD45RA⁺, T CM: CD62L⁺ CD45RA⁻, T EM: CD62L⁻ CD45RA⁻, and TEMRA: CD62L⁻ CD45RA⁺. CD56dim NK cells that did not stain positive for CD158b, CD158f, CD158e/k or NKG2A were considered unlicensed.

Table 2
CMV effect on CD56dim NK cell responses and on CD56bright/CD56dim ratio.

| Analyte   | Stimulus | CMV  | Mean | SEM  | Sig  |
|-----------|----------|------|------|------|------|
| CD107a    | Nil      | –    | 3.91 | 0.52 | .808 |
|           | +        | 4.12 | 0.50 |      |      |
| K562      | –        | 15.89| 1.80 | .937 |
|           | +        | 15.72| 1.15 |      |      |
| NKp46     | –        | 20.50| 3.43 | .755 |
|           | +        | 21.84| 2.30 |      |      |
| IFN-γ     | Nil      | –    | 1.31 | 0.24 | .928 |
|           | +        | 1.34 | 0.20 |      |      |
| K562      | –        | 3.46 | 0.64 | .070 |
|           | +        | 2.32 | 0.30 |      |      |
| NKp46     | –        | 9.33 | 1.47 | .920 |
|           | +        | 9.11 | 1.23 |      |      |
| IL-2      | –        | 2.00 | 0.37 | .814 |
|           | +        | 2.12 | 0.28 |      |      |
| IL-15     | –        | 2.63 | 0.48 | .342 |
|           | +        | 3.94 | 0.83 |      |      |
| IL-12/18  | –        | 20.55| 4.67 | .399 |
|           | +        | 16.54| 2.32 |      |      |
| MIP-1β    | Nil      | –    | 6.99 | 0.86 | .048 |
|           | +        | 5.06 | 0.49 |      |      |
| K562      | –        | 32.98| 3.28 | .599 |
|           | +        | 30.93| 2.05 |      |      |
| NKp46     | –        | 48.92| 5.82 | .725 |
|           | +        | 46.39| 3.84 |      |      |
| IL-2      | –        | 40.14| 3.71 | .123 |
|           | +        | 33.78| 2.08 |      |      |
| IL-15     | –        | 60.90| 4.11 | .450 |
|           | +        | 56.99| 2.76 |      |      |
| IL-12/18  | –        | 31.40| 5.28 | .420 |
|           | +        | 27.06| 2.63 |      |      |
| Ratio*    | Nil      | –    | .0476| .0073| .960 |
|           | +        | .0471| .0046|      |      |

CD56dim NK cells were gated and tested for the indicated analyte in response to the designated stimulus. Shown is the mean and standard error of the mean (SEM) for subjects who were CMV infected (+) or not CMV infected (–). Bold print denotes significant mean differences as determined by student’s T test.

* CD56bright/CD56dim ratio, tested immediately ex vivo.
2.2. NK cell stimulation

Peripheral blood mononuclear cells (PBMC, \(0.5 \times 10^6\)) were incubated in 6-well plates in media (RPMI 1640 media, 10% FBS, 20 mM glutamine, non-essential amino acids, and antibiotics) in the presence of either no stimulation, IL-2 (200,000 U/L, Biological Resources Branch, National Cancer Institute, Frederick, MD), IL-15 (100 μg/L, BioLegend), IL-12 (10 μg/L, Peprotech, Rocky Hill, NJ) plus IL-18 (100 μg/L, R&D Systems, Minneapolis, MN), overnight, or with \(1 \times 10^6\) K562 cells (E:T ratio 1:2) for 3 h at 37 °C. The final three hours of incubation were in the presence of 5 mg/L brefeldin A and 2 μM monensin (BioLegend). Cells were then washed, and stained with CD3, CD16, CD56 and CD107a (as described above), fixed in 2% paraformaldehyde solution, then permeabilized (1x Permeabilization buffer, eBioscience) and stained with anti-IFN-γ and anti-MIP-1β mAb. For anti-NKp46 stimulation, wells in 24-well plates were coated by incubation with 0.5 mL 2.5 mg/L anti-NKp46 mAb (eBioscience) in PBS overnight at 4 °C. Unbound mAb was removed by washing with PBS. Cells were cultured overnight at 37 °C in 5% CO2 with 500 ng/L IL-12, transferred to anti-NKp46-coated wells, cultured for 3 h, harvested, and stained as above.

### Table 3

| Analyte        | Sex | Mean  | SEM  | P value |
|----------------|-----|-------|------|---------|
| CRP            | ♂   | 2.824 | 0.463| .173    |
|                | ♀   | 1.973 | 0.408|         |
| IL-15          | ♂   | 4.718 | 0.210| .462    |
|                | ♀   | 4.527 | 0.152|         |
| Adiponectin    | ♂   | 21.47 | 2.07 | .019    |
|                | ♀   | 14.13 | 2.19 |         |
| S1P            | ♂   | 754.2 | 29.32| .642    |
|                | ♀   | 732.2 | 36.3 |         |
| dhS1P          | ♂   | 65.30 | 2.90 | .536    |
|                | ♀   | 62.70 | 3.00 |         |
| S1P+dhS1P      | ♂   | 819.5 | 31.21| .625    |
|                | ♀   | 794.9 | 38.4 |         |
| RBP4           | ♂   | 45.80 | 1.06 | .039    |
|                | ♀   | 49.02 | 1.08 |         |
| 25(OH)D        | ♂   | 38.35 | 2.78 | .025    |
|                | ♀   | 30.21 | 2.16 |         |
| Vitamin D Supplement | ♂   | .625 | .101 | .002    |
|                | ♀   | .192 | .079 |         |

For each tested analyte, the mean and standard error of the mean (SEM) is shown for each gender. Analytes and their units are C-reactive protein (CRP, mg/L), IL-15 (mg/L), adiponectin (mg/L), sphingosine-1-phosphate (S1P, nM), dihydroS1P (dhS1P, nM), the sum of S1P and dhS1P (nM), RBP4 (mg/L), and vitamin D (25(OH)D, μg/L). Bold print denotes significant mean differences as determined by student’s T test.
2.3. Cytokines, soluble immune mediators, and antibodies

Following manufacturer instructions, enzyme-linked immunosorbent assay was used to measure plasma C-reactive protein (CRP) and adiponectin levels (eBioscience) and retinol-binding protein 4 (RBP4) levels (Abcam, Cambridge, England). Plasma IL-15 was quantified with the QuantiGlo Chemiluminescent Immunoassay kit (R&D Systems). Reported values are the average of a single measurement of each serum sample tested in two (IL-15), three (Adiponectin, CRP), or four (RBP4) independent experiments. For sphingosine-1-phosphate (S1P) and dihydrosphingosine-1-phosphate (dhS1P) measurements, plasma samples with added deuterated standards [2] were extracted using acidified organic solvents [3]. S1P and dhS1P levels were measured using high pressure liquid chromatography–electrospray ionization tandem mass spectrometry, and quantified by comparing levels to the internal standard [4]. The University of Kentucky Clinical Chemistry Laboratory measured serum 25(OH)D by liquid chromatography-tandem mass spectrometry using atmospheric pressure chemical ionization in positive ion mode and quantitated by comparison to a deuterated internal standard. Plasma samples were diluted 100-fold and anti-cytomegalovirus (CMV) IgG was measured in duplicate via binding to immobilized CMV antigen. Bound CMV IgG was detected by horseradish peroxidase conjugated anti-human IgG antibody (DRG CMV IgG ELISA, Springfiled, NJ). None of the positive or negative anti-CMV IgG levels were close to the intermediate range.

Table 4
Correlation of RBP4 levels with NK cell responses.

| NK Cell | Stimulus | Analyte      | ρ     | Sig. |
|---------|----------|--------------|-------|------|
| CD56bright | None | CD107a       | .025  | .864 |
| CD56bright | K562 | CD107a       | .249  | .082 |
| CD56bright | NKp46 | CD107a       | -.303 | .033 |
| CD56dim | None | CD107a       | .018  | .901 |
| CD56dim | K562 | CD107a       | .065  | .656 |
| CD56dim | NKp46 | CD107a       | -.221 | .123 |
| CD56bright | None | IFN-γ       | -.065 | .655 |
| CD56bright | K562 | IFN-γ       | .259  | .069 |
| CD56bright | NKp46 | IFN-γ       | -.334 | .018 |
| CD56bright | IL-2 | IFN-γ       | -.223 | .120 |
| CD56bright | IL-15 | IFN-γ       | -.180 | .212 |
| CD56bright | IL-12/18 | IFN-γ | -.228 | .111 |
| CD56dim | None | IFN-γ       | .038  | .795 |
| CD56dim | K562 | IFN-γ       | .142  | .325 |
| CD56dim | NKp46 | IFN-γ | -.308 | .030 |
| CD56dim | IL-2 | IFN-γ       | -.022 | .879 |
| CD56dim | IL-15 | IFN-γ       | .194  | .177 |
| CD56dim | IL-12/18 | IFN-γ | -.234 | .101 |
| CD56bright | None | MIP-1β      | -.177 | .219 |
| CD56bright | K562 | MIP-1β      | .253  | .076 |
| CD56bright | NKp46 | MIP-1β      | .246  | .086 |
| CD56bright | IL-2 | MIP-1β      | .336  | .017 |
| CD56bright | IL-15 | MIP-1β      | .238  | .096 |
| CD56bright | IL-12/18 | MIP-1β | -.131 | .363 |
| CD56dim | None | MIP-1β      | -.262 | .066 |
| CD56dim | K562 | MIP-1β      | -.089 | .537 |
| CD56dim | NKp46 | MIP-1β      | -.300 | .034 |
| CD56dim | IL-2 | MIP-1β      | -.012 | .933 |
| CD56dim | IL-15 | MIP-1β      | -.127 | .379 |
| CD56dim | IL-12/18 | MIP-1β | -.284 | .046 |

Shown are nonparametric Spearman’s correlation coefficients (ρ) and significance (Sig.) between RBP4 level and the indicated parameter. Bold print denotes significant correlations.
Table 5
The influence of Adiponectin, RBP4, and 25(OH)D levels on NK cell responses does not outweigh sex effect.

| Model | Unstandardized coefficients | Standardized coefficients | t   | Sig. |
|-------|-----------------------------|---------------------------|-----|------|
|       | B                           | Std. error                | Beta |      |
|       |                             |                           |      |      |
|       | Dependent Variable: % CD107a on K562-stimulated CD56dim NK cells | | | |
| 1     | (Constant)                  | 17.971                    | 1.324 | 13.574 | .000 |
|       | Sex                         | -4.232                    | 1.836 | -.316  | -.230 | .026 |
| 2     | (Constant)                  | 18.462                    | 2.324 | -2.316 | 7.944 | .000 |
|       | Sex                         | -4.400                    | 1.965 | -2.328 | 2.240 | .030 |
|       | Adiponectin                 | -.023                     | .089  | -.038  | -.258 | .797 |
|       | Dependent Variable: % CD107a on K562-stimulated CD56dim NK cells | | | |
| 1     | (Constant)                  | 17.971                    | 1.324 | 13.574 | .000 |
|       | Sex                         | 4.232                     | 1.836 | .316   | 2.305 | .026 |
| 2     | (Constant)                  | 9.696                     | 8.085 | -2.278 | 1.199 | .236 |
|       | Sex                         | -4.814                    | 1.918 | -2.349 | -2.510 | .016 |
|       | Adiponectin                 | .181                      | .174  | .148   | 1.037 | .305 |
|       | Dependent Variable: % CD107a on K562-stimulated CD56dim NK cells | | | |
| 1     | (Constant)                  | 9.696                     | 8.085 | 1.199  | .236 |
|       | Sex                         | 4.814                     | 1.918 | .359   | 2.240 | .030 |
| 2     | (Constant)                  | 19.254                    | 3.337 | -2.278 | 1.199 | .236 |
|       | Sex                         | -4.332                    | 1.982 | -2.349 | -2.510 | .016 |
|       | Adiponectin                 | .256                      | .176  | .148   | 1.037 | .305 |
|       | Dependent Variable: % CD107a on K562-stimulated CD56dim NK cells | | | |
| 1     | (Constant)                  | 19.254                    | 3.337 | -2.278 | 1.199 | .236 |
|       | Sex                         | 4.332                     | 1.982 | .359   | 2.240 | .030 |
| 2     | (Constant)                  | 9.696                     | 8.085 | 1.199  | .236 |
|       | Sex                         | -4.814                    | 1.918 | -2.349 | -2.510 | .016 |
|       | Adiponectin                 | .023                      | .089  | -.038  | -.258 | .797 |
|       | Dependent Variable: % IFN-γ positive on NKp46-stimulated CD56dim NK cells | | | |
| 1     | (Constant)                  | 11.182                    | 1.354 | 8.255  | .000 |
|       | Sex                         | -3.942                    | 1.860 | -3.01  | -2.120 | .040 |
| 2     | (Constant)                  | 11.182                    | 1.354 | 8.255  | .000 |
|       | Sex                         | -3.870                    | 1.878 | -2.85  | -2.060 | .045 |
|       | Adiponectin                 | .074                      | .090  | .121   | .822  | .415 |
|       | Dependent Variable: % IFN-γ positive on NKp46-stimulated CD56dim NK cells | | | |
| 1     | (Constant)                  | 11.182                    | 1.354 | 8.255  | .000 |
|       | Sex                         | -3.870                    | 1.878 | -2.85  | -2.060 | .045 |
| 2     | (Constant)                  | 11.433                    | 1.409 | 8.112  | .000 |
|       | Sex                         | -3.862                    | 1.972 | -2.80  | -1.958 | .056 |
|       | Adiponectin                 | .074                      | .090  | .121   | .822  | .415 |
|       | Dependent Variable: % IFN-γ positive on NKp46-stimulated CD56dim NK cells | | | |
| 1     | (Constant)                  | 11.433                    | 1.409 | 8.112  | .000 |
|       | Sex                         | -3.862                    | 1.972 | -2.80  | -1.958 | .056 |
| 2     | (Constant)                  | 9.594                     | 2.362 | 4.061  | .000 |
|       | Sex                         | -3.047                    | 1.942 | -2.24  | -1.569 | .123 |
|       | RBP4                         | -.256                     | .176  | -.207  | -1.449 | .154 |
|       | Dependent Variable: % IFN-γ positive on NKp46-stimulated CD56dim NK cells | | | |
| 1     | (Constant)                  | 9.594                     | 2.362 | 4.061  | .000 |
|       | Sex                         | -3.047                    | 1.942 | -2.24  | -1.569 | .123 |
| 2     | (Constant)                  | 10.902                    | 3.600 | 3.032  | .004 |
|       | Sex                         | -2.110                    | .854  | -2.470 | .017  |
|       | RBP4                         | -.089                     | .078  | -.158  | -1.141 | .260 |
|       | Dependent Variable: % MIP-1β positive on unstimulated CD56dim NK cells | | | |
| 1     | (Constant)                  | 6.849                     | .591  | 11.591 | .000 |
|       | Sex                         | -2.395                    | .819  | -2.93  | -2.923 | .005 |
| 2     | (Constant)                  | 6.766                     | 1.038 | 6.520  | .000 |
|       | Sex                         | -2.367                    | .877  | -2.697 | .010  |
|       | Adiponectin                 | .004                      | .040  | .014   | .097  | .923 |
|       | Dependent Variable: % MIP-1β positive on unstimulated CD56dim NK cells | | | |
| 1     | (Constant)                  | 6.849                     | .591  | 11.591 | .000 |
|       | Sex                         | -2.395                    | .819  | -2.93  | -2.923 | .005 |
| 2     | (Constant)                  | 10.902                    | 3.600 | 3.032  | .004 |
|       | Sex                         | -2.110                    | .854  | -2.470 | .017  |
|       | RBP4                         | -.089                     | .078  | -.158  | -1.141 | .260 |
|       | Dependent Variable: % MIP-1β positive on unstimulated CD56dim NK cells | | | |
| 1     | (Constant)                  | 6.777                     | .615  | 11.186 | .000 |
|       | Sex                         | -2.277                    | .860  | -2.647 | .011  |
| 2     | (Constant)                  | 5.671                     | 1.537 | 3.689  | .001  |
|       | Sex                         | -2.021                    | .913  | -2.213 | .032  |
| Model | Unstandardized coefficients | Standardized coefficients | t     | Sig. |
|-------|-----------------------------|---------------------------|-------|------|
|       | B          | Std. error | Beta  |       |       |
| 25(OH)D | .031       | .037       | .126  | .857 | .396 |

### Dependent Variable: % MIP-1β positive on K562-stimulated CD56dim NK cells

|       | B          | Std. error | Beta  |       |       |
|-------|-------------|------------|-------|-------|-------|
| 1     | 35.617      | 2.380      | .126  | 14.965| .000  |
| 2     | 32.893      | 2.380      | .126  | 14.965| .000  |

### Dependent Variable: % MIP-1β positive on K562-stimulated CD56dim NK cells

|       | B          | Std. error | Beta  |       |       |
|-------|-------------|------------|-------|-------|-------|
| 1     | 34.600      | 2.380      | .126  | 14.965| .000  |
| 2     | 31.242      | 2.380      | .126  | 14.965| .000  |

### Dependent Variable: % MIP-1β positive on NKp46-stimulated CD56dim NK cells

|       | B          | Std. error | Beta  |       |       |
|-------|-------------|------------|-------|-------|-------|
| 1     | 54.117      | 2.380      | .126  | 14.965| .000  |
| 2     | 105.250     | 2.380      | .126  | 14.965| .000  |

### Dependent Variable: % MIP-1β positive on NKp46-stimulated CD56dim NK cells

|       | B          | Std. error | Beta  |       |       |
|-------|-------------|------------|-------|-------|-------|
| 1     | 54.117      | 2.380      | .126  | 14.965| .000  |
| 2     | 105.250     | 2.380      | .126  | 14.965| .000  |

### Dependent Variable: % MIP-1β positive on IL-15-stimulated CD56dim NK cells

|       | B          | Std. error | Beta  |       |       |
|-------|-------------|------------|-------|-------|-------|
| 1     | 63.646      | 2.380      | .126  | 14.965| .000  |
| 2     | 62.546      | 2.380      | .126  | 14.965| .000  |

### Dependent Variable: % MIP-1β positive on IL-15-stimulated CD56dim NK cells

|       | B          | Std. error | Beta  |       |       |
|-------|-------------|------------|-------|-------|-------|
| 1     | 63.646      | 2.380      | .126  | 14.965| .000  |
| 2     | 62.546      | 2.380      | .126  | 14.965| .000  |
2.4. Statistical methods

For means comparisons, data were first analyzed with Levene’s test for equality of variance using SPSS, version 22 (IBM, Armonk, NY). When gender based variances were significantly unequal, means were compared by the nonparametric Mann–Whitney U test. None of the means analyzed by the Mann–Whitney test significantly differed by gender. Otherwise, means were compared using the 2-tailed student’s t test. Levels are displayed as means ± SEM.

Acknowledgments

We thank Jody Clasey, Doug Long, Kim Holt, Andrew J. Morris, Maja Redzic, Yuri M. Klyachkin, and CRO nurses for help with study subjects and data analysis. We thank Suzanne Segerstrom and Philip Westgate for statistical advice. This work was supported by NIH grant, AG040542, and by University of Kentucky Center for Clinical and Translational Science Analytics Lab and CTSA funding UL1TR000117.

Transparency document. Supplementary material

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.dib.2016.06.006.

Table 5 (continued)

| Model       | Unstandardized coefficients | Standardized coefficients | t    | Sig.    |
|-------------|-----------------------------|---------------------------|------|---------|
|             | B              | Std. error | Beta |        |        |
| Sex         | –8.637         | 4.751       | –0.278 | –1.818 | .076   |
| 25(OH)D     | .016           | .191        | .013  | .083   | .934   |

Multivariate analysis of sex-specific NK cell responses. Each dependent variable (NK cell response) was compared with the independent variable, sex (Model 1, Pearson correlation), or with sex along with other independent variables, either adiponectin, RBP4, or 25(OH)D (vitamin D) plasma levels (Model 2, multiple linear regression). For scoring purposes, males = 1; females = 0. Therefore, a negative correlation with sex indicates stronger responses in women than in men. 25(OH)D levels were not available for 1 female and 2 male subjects. Therefore, the significance (Sig.) of the correlation between sex and the NK cell response is different than when adiponectin and RBP4 effects are analyzed. The value of the unstandardized coefficient (B) reflects the amount of change in the predicted preference ranking. Using the standardized coefficient (Beta), interpretations are based on the standard deviation (SD) of the variable. Each standardized coefficient (Beta) indicates the number of SD that the dependent variable changes for a 1 SD change in the independent variable, the other independent variable remaining constant. For both B and Beta, the higher the absolute value (positive or negative), the greater the effect of the given independent variable on the dependent variable. For example, in the first table (Model 2), the absolute value of B and Beta for Sex was significantly greater than B and Beta for Adiponectin, indicating that Sex had a stronger (inverse) correlation with the NK cell response than did Adiponectin. Significant values (as determined by Pearson correlation, Model 1, and multiple linear regression, Model 2) are shown in bold print.

References

[1] A. Al-Attar, S.R. Presnell, C.A. Peterson, D.T. Thomas, C.T. Lutz, The effect of sex on immune cells in healthy aging: elderly women have more robust natural killer lymphocytes than do elderly men, Mech. Ageing Dev. Rev. (2016).
[2] D. Siow, M. Sunkara, T.M. Dunn-Giroux, A.J. Morris, B. Wattenberg, ORMDL/serine palmitoyltransferase stoichiometry determines effects of ORMDL3 expression on sphingolipid biosynthesis, J. Lipid Res. (2015).
[3] T.P. Mathews, A.J. Kennedy, Y. Kharel, P.C. Kennedy, O. Nicoara, M. Sunkara, A.J. Morris, B.R. Wamhoff, K.R. Lynch, T.L. Macdonald, Discovery, biological evaluation, and structure-activity relationship of amidine based sphingosine kinase inhibitors, J. Med. Chem. 53 (2010) 2766–2778.
[4] S. Selim, M. Sunkara, A.K. Salous, S.W. Leung, E.V. Berdyshhev, A. Bailey, C.L. Campbell, R. Charnigo, A.J. Morris, S.S. Smyth, Plasma levels of sphingosine 1-phosphate are strongly correlated with haematocrit, but variably restored by red blood cell transfusions, Clin. Sci. 121 (2011) 565–572.