

Changes in immune Cell Types in Breast Suggest a Decline in Immune Surveillance and Increased Immunosuppression with Age

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Research article

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Abstract

Background : A majority of breast cancers (BC) are age-related and we seek to determine what cellular and molecular changes occur in breast tissue with age that make women more susceptible to cancer initiation. Immune-epithelial cell interactions are important during mammary gland development and the immune system plays an important role in BC progression. The composition of human immune cell populations is known to change in peripheral blood with age and in breast tissue during BC progression. Less is known about changes in immune populations in normal breast tissue and how their interactions with mammary epithelia change with age.

Methods : We quantified densities of T cells, B cells, and macrophage subsets in pathologically normal breast tissue from 122 different women who ranged in age from 24 to 74 years old. Donor-matched peripheral blood from a subset of 20 donors was analyzed by flow cytometry. Tissue immune cell densities and localizations relative to the epithelium were quantified in situ with machine learning-based analyses of multiplex immunohistochemistry-stained tissue sections. In situ results were corroborated with flow cytometry analyses of peri-epithelial immune cells from primary organoid preparations and transcriptome analyses of public data from bulk tissue reduction mammaplasties.

Results : Proportions of immune cell subsets in breast tissue and donor-matched peripheral blood were not correlated. Density (cells/mm²) of T and B lymphocytes in situ decreased with age. T cells and macrophages preferentially localized near or within epithelial bilayers, rather than the intralobular stroma. M2:M1 macrophage ratio increased with age and was accompanied by an increased density of M2 in the intralobular stroma. Transcriptional signature analyses suggested age-dependent decline in adaptive immune cell populations and functions and increased innate immune cell activity.

Conclusions : T cells and macrophages are so intimately associated with the epithelia that they are embedded within the bilayer, suggesting an important role for immune-epithelial cell interactions. Age-associated decreased T cell density in peri-epithelial regions, and increased M2 macrophage density in intralobular stroma suggests the emergence of a tissue microenvironment that is simultaneously immune-senescent and immunosuppressive with age .

Full Text

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Figures

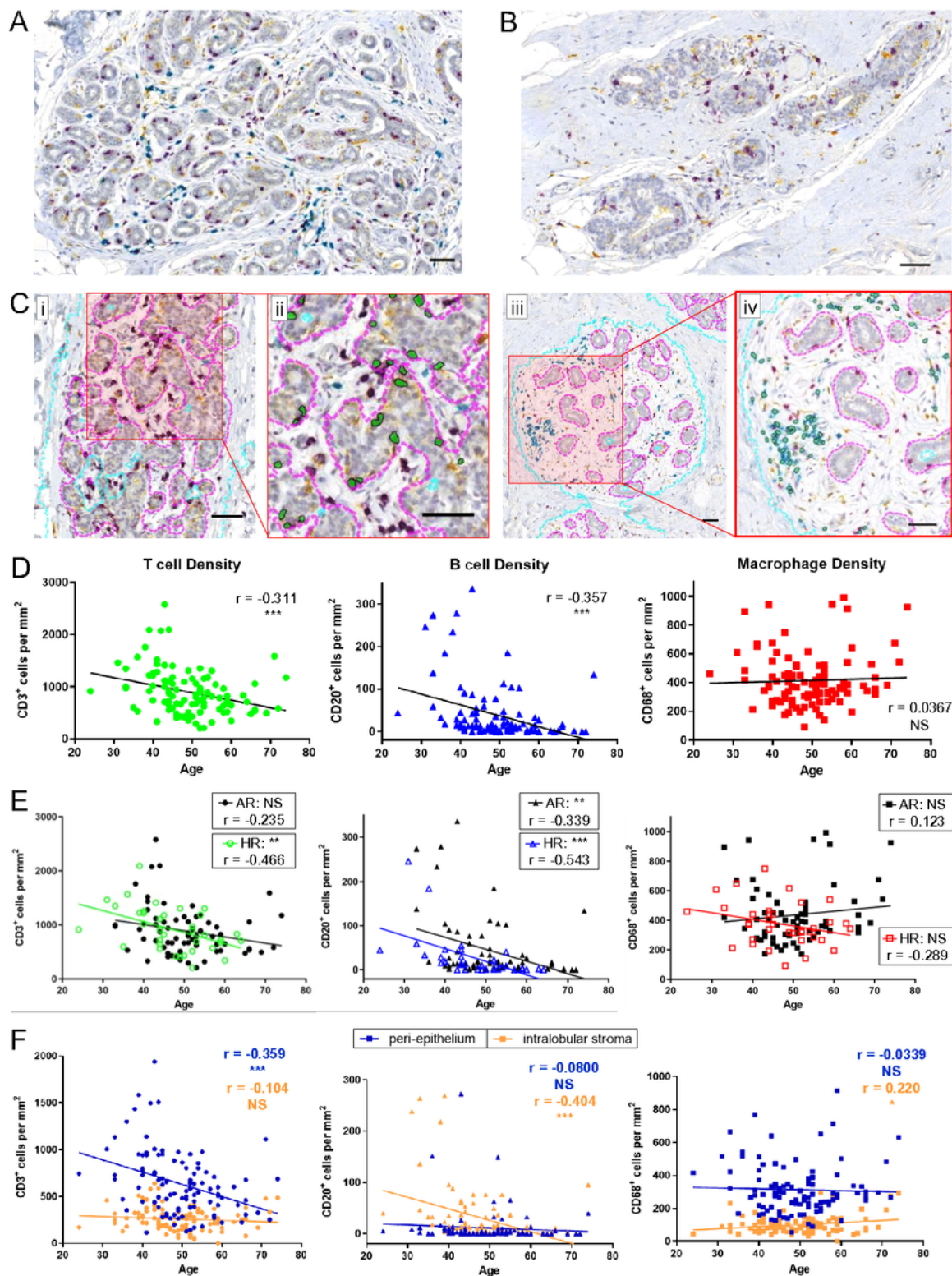


Figure 1

immune cells closely associated with mammary epithelium and their density in situ changed with age. Representative images of IHC triple-stained pathologically normal breast tissue from (A) young (33y) and (B) older (66y) donors. Purple (CD3), Teal (CD20), Yellow (CD68). Scalebars are 50µm. (C) Machine-learning algorithms were used to first segment the tissue into fat and stroma regions (FSR) or epithelium-enriched regions (EER) (light blue dotted lines), and then to further segment tissue within EER as

intralobular stroma (ILS) or peri-epithelium (PE) (magenta dotted lines). The three immune cell types were then classified within EER as being either within the PE (within magenta dotted lines) or within the ILS (within light blue and outside of magenta dotted lines) and quantified in each area. (i) and (iii) show segmentation output only, (ii) shows cell classification output of CD3+ T cells within PE from inset in (i), and (iv) shows cell classification output of CD20+ B cells within ILS from inset in (iii). Scalebars are 50µm. (D) Total density (cells per mm²) of each immune cell type in EER was quantified in situ and compared with donor age by linear regression (n=102). r, correlation coefficient, and p-value of each regression are indicated. (E) Age regressions of each immune cell type when samples are grouped into AR (filled shapes; n=67) and HR (open shapes; n=36). r and p-value of each regression are indicated. (F) Immune cell densities (cells per mm²) quantified in PE (blue) and in ILS and distant from the epithelium (orange) (n=102). r and p-value of each regression are indicated. * p<0.05, ** p<0.01, *** p<0.001, NS=not significant (P>0.05).

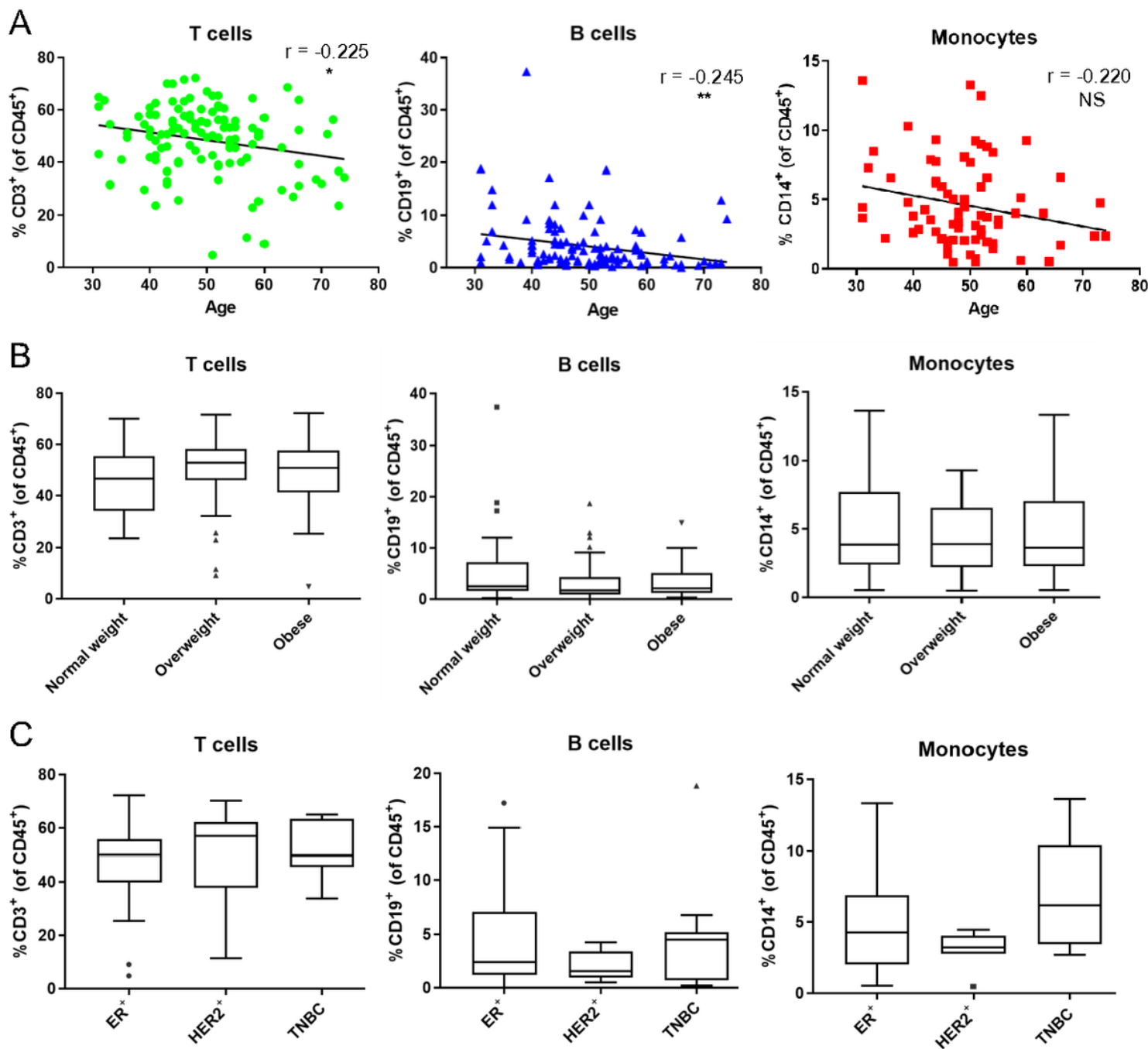


Figure 2

Mammary gland peri-epithelial immune cells were isolated and measured by flow cytometry. (A) Immune cell proportions quantified via flow cytometry and compared with donor age by linear regression. T cells (CD3+) (n=116), B cells (CD19+) (n=116), Monocytes (CD14+) (n=74). r and p -value of each regression are indicated. (B) ANOVA for immune cell type and BMI (n=115): normal weight ($18.5 \geq \text{BMI} < 25$) (n=37), overweight ($25 \geq \text{BMI} < 30$) (n=43), obese ($\text{BMI} \geq 30$) (n=35). (C) ANOVA for immune cell type and tumor receptor status in contralateral tumor tissue (n=78): ER+ (n=53), HER2+ (n=14), TNBC (n=11). All ANOVA p -values were NS.

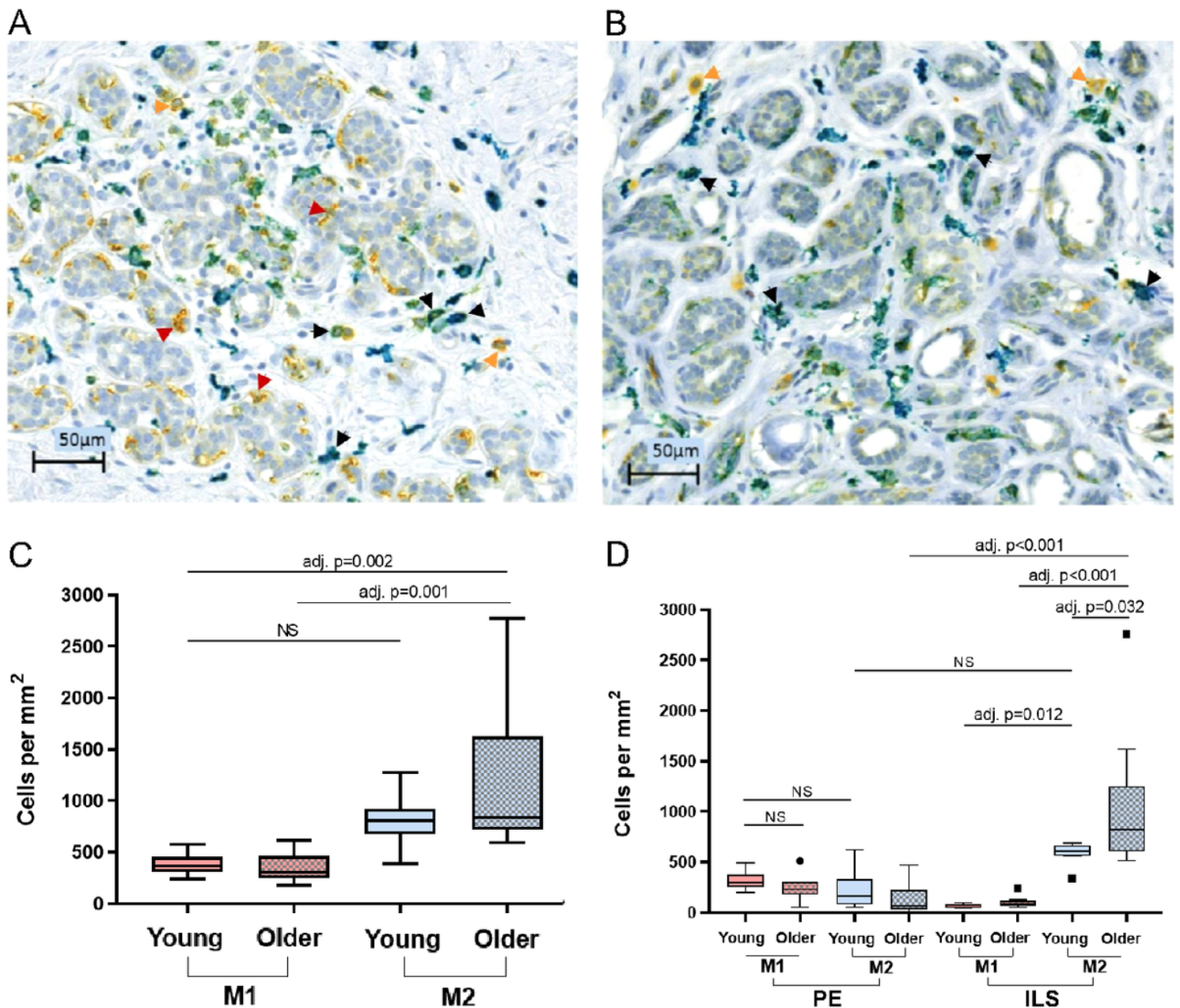


Figure 3

M1 and M2 macrophage quantification in IHC-stained breast tissue further characterized the aging immune phenotype. Representative images of IHC double-stained breast tissue from (A) young and (B) older donors. Yellow (CD68+), Teal (CD163+), Green (CD163+/CD68+). M1 (CD68+/CD163-) and M2 (CD163+/CD68- and CD163+/CD68+). Red arrowheads point to peri-epithelial (PE) M1 macrophages, orange arrowheads point to intralobular stromal (ILS) M1 macrophages, and black arrowheads point to ILS M2 macrophages. Scalebars are 50µm. (C) Density of M1 and M2 macrophages quantified in situ from young (≤41y, n=8) and older (≥58y, n=9) donor age groups. (D) Density of M1 and M2 macrophages in PE or ILS in each age group. Adjusted p-values (adj. p-val) of ANOVA post-hoc analyses between age groups are indicated.

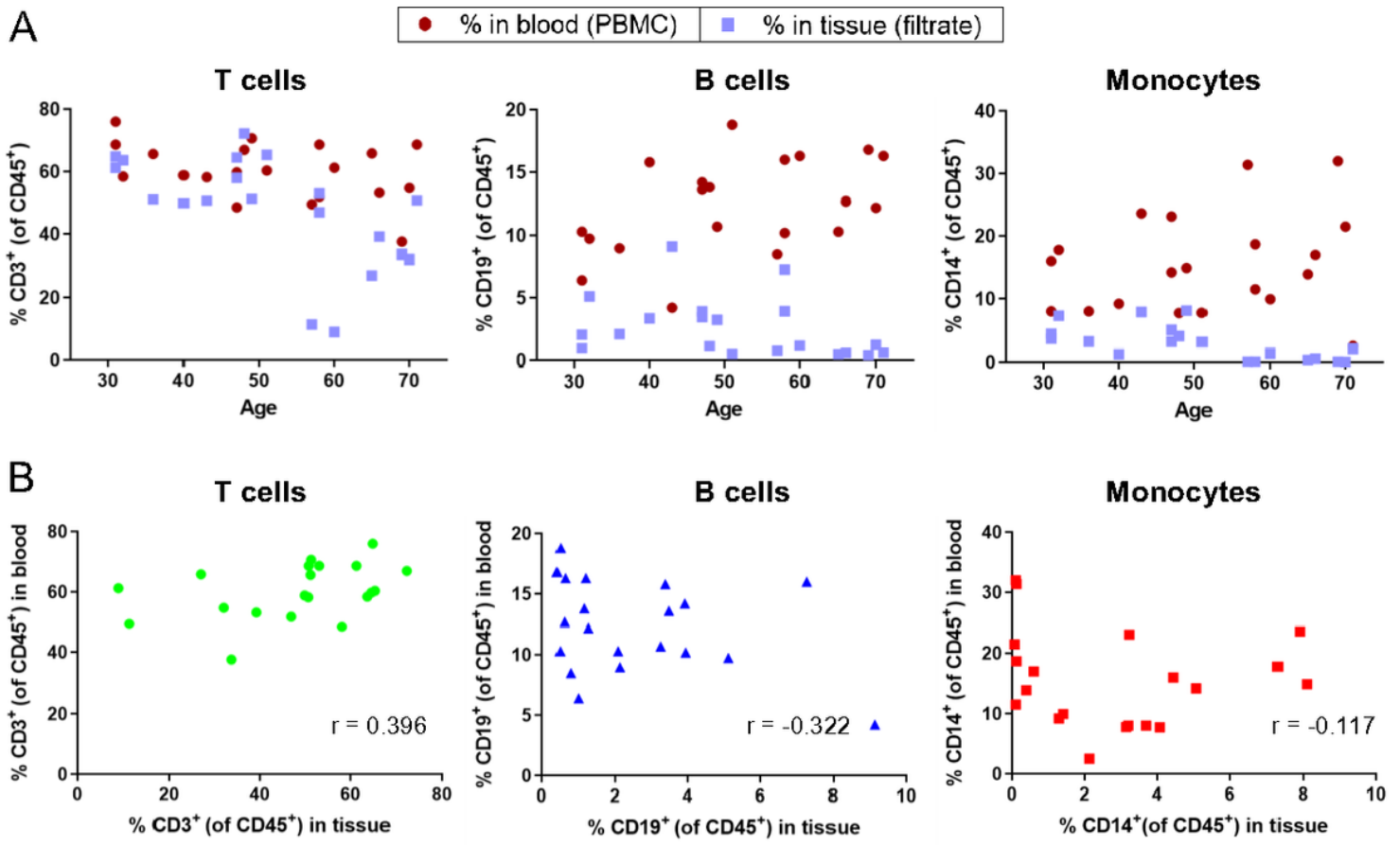


Figure 4

Immune cell proportions were not correlated in donor-matched peripheral blood and breast tissue. Donor-matched PBMCs (n=20) were analyzed by flow cytometry in the same manner as filtrates to determine proportions of immune cells. (A) Percent of each immune cell type in blood (red circles) and in tissue (blue squares) compared with donor age. (B) Pearson's correlation analysis for each cell type in blood and tissue. r , Pearson's correlation coefficient, is indicated. P-values were all NS.

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