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Research Article

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Is Chronic Exposure to Low-Dose Organochlorine Pesticides a New Risk Factor of T-cell Immunosenescence?

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Abstract

Background: T-cell immunosenescence, a hallmark of an aging immune system, is potentially linked to the risk of developing cancer and other aging-related diseases. Chronic infection by cytomegalovirus (CMV) has been widely studied as a risk factor for T-cell immunosenescence, but the role of persistent chemicals has never been examined. As a typical example of persistent chemicals, we evaluated whether organochlorine pesticides (OCPs) are related to T-cell immunosenescence in the general population.

Methods: Serum concentrations of β-hexachlorocyclohexane, *p,p′*-DDT, *p,p′*-DDE, and trans-nonachlor were measured in 95 Korean adults ages 30 to 64 years. T-cell immunosenescence was assessed by the frequencies of $CD8^+CD57^+$, $CD8^+CD28^-$, $CD4^+CD57^+$, and $CD4^+CD28^-$ T lymphocytes in 20 mL of fresh peripheral blood.

Results: The senescence of $CD8^+$ T lymphocytes was the most consistently associated with OCPs. For quartiles of measurements of OCPs, adjusted mean percentages of $CD8^+CD57^+$ and $CD8^+CD28^-$ T lymphocytes in the $CD8^+$ T lymphocyte population were 23.9, 27.6, 31.0, and 38.7 ($P_{trend} < 0.01$) and 25.6, 27.3, 28.0, and 35.5 ($P_{trend} = 0.02$), respectively. When we compared the strength of the associations among OCPs, CMV IgG titer, and age, OCPs showed the strongest association with markers of immunosenescence. Importantly, the association between OCPs and immunosenescence markers was more prominent among participants without known risk factors, such as a young age or low CMV immunoglobulin G titer.

Conclusions: Chronic exposure to low-dose OCPs may be a new risk factor for T-cell immunosenescence.

Impact: T-cell immunosenescence may be one possible mechanism linking low-dose OCPs and many chronic diseases.

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Introduction

Functional alteration in the immune system during aging is generally referred to as "immunosenescence," which has been potentially linked to the risk of developing many age-related conditions, such as cancer and the increased susceptibility to infectious diseases (1–3).

Although immunosenescence can be considered an intrinsic aging-related change, there is substantial variation among individuals. At present, the most well-known environmental risk factor affecting immunosenescence is chronic infection by pathogens, such as cytomegalovirus (CMV; ref. 4).

As immune system is one of the targets of xenobiotic-induced toxicity (5), similar to chronic infections, chronic exposure to persistent chemicals may play a role in the process of immunosenescence. However, the effect of chronic exposure to persistent chemicals on immunosenescence has not yet been evaluated in either human or experimental studies. Typical examples of persistent chemicals include persistent organic pollutants (POPs), strong lipophilic chemicals that are mainly stored in adipose

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tissue and continuously released into the circulation through lipolysis (6).

There is growing evidence that low-dose chlorinated POPs, especially organochlorine pesticides (OCPs), may be important in the development of many obesity-related diseases (6, 7). As many pesticides decrease immune-competence or cause inappropriate immune-stimulation *in vitro* and/or *in vivo* (8), immunosenescence may also be a possible adverse effect of OCPs.

Importantly, due to wide contamination of the food chain since the mid-20th century, the most important external sources of OCP exposure are currently fatty animal foods (9). OCP-contaminated human adipose tissue also plays a role as an internal source of exposure. In fact, OCP serum concentration in contemporary humans is considered a surrogate marker of lipophilic chemical mixtures contained in adipose tissue (10).

Although there is no standardized set of parameters to determine the status of immunosenescence in humans, one hallmark feature of immunosenescence is a low number and percentage of naïve T lymphocytes, especially $CD8^+$ T lymphocytes expressing CD28, and the accumulation of late-differentiated $CD8^+$ T lymphocytes expressing CD57 (11). This is likely due to the conversion of naïve $CD8^+$ T cells to memory $CD8^+$ T cells by chronic exposure to immune challenges (11). In addition to $CD8^+$ T lymphocytes, the loss of CD28 and the gain of CD57 expression is also observed with $CD4^+$ T lymphocytes during chronic immune activation (12), although the $CD4^+$ T-cell population accumulates at a significantly lower rate than that of $CD8^+$ T lymphocytes (13).

In this study, we evaluated whether serum concentrations of OCPs were associated with immunosenescence in human participants using $CD8^+CD28^-$, $CD8^+CD57^+$, $CD4^+CD28^-$, or $CD4^+CD57^+$ T-cell populations as markers of immunosenescence. In particular, we compared these associations to those of well-established risk factors of immunosenescence, that is, age and CMV infection.

Materials and Methods

Study participants

A total of 100 participants ages 30 to 69 years without any history of cancer, myocardial infarction, stroke, or heart failure were recruited at the Routine Health Check-up Center of Kyungpook National University Hospital, Daegu, Korea, from October 2013 to December 2013. After excluding five participants who did not provide enough fresh blood (20 mL) for immunophenotyping, the final sample size was 95. Written informed consent was obtained from all participants. This study was conducted with the approval of the institutional review board at the Kyungpook National University Hospital.

Demographic information and lifestyle factors were determined for all the participants by trained interviewers using a standardized questionnaire. Height and body weight were measured using standard methods in light clothes. Body mass index (BMI) was calculated as weight divided by height squared (kg/m^2). Blood samples were obtained by venipuncture in the morning after overnight fasting. Total cholesterol, triglycerides, HDL-cholesterol, and fasting glucose were determined by enzymatic methods using ADVIA 1650 (Bayer Inc.). CMV IgG antibody levels were determined using a chemiluminescent enzyme immunoassay (IMMULITE 2000XPi; Siemens).

Immunophenotyping of peripheral blood mononuclear cells

Peripheral blood mononuclear cells (PBMC) were isolated from whole blood using Ficoll-Hypaque (GE Healthcare) and immediately analyzed for CD57 and CD28 expression. One hundred thousand cells were stained with fluorochrome-conjugated monoclonal antibodies against surface antigens in PBS for 20 minutes at 4°C. The antibodies used included anti-CD3-Horizon V500 (clone UCHT1), anti-CD4-PE-Cy7 (clone RPA-T4), anti-CD8-APC-H7 (clone SK1), anti-CD28-APC (clone CD28.2; all from BD Biosciences), and anti-CD57-eFluor 450 (clone TB01; eBioscience). Multicolor flow cytometry was performed using an LSR II instrument (BD Biosciences), and compensation was performed using UltraComp eBeads (eBioscience) and FACSDiva software (BD Biosciences). FlowJo software (Treestar) was used to analyze the data.

Measurements of OCPs

OCPs levels were measured in serum by high-resolution gas chromatography/high-resolution mass spectrometry using isotope dilution for quantification. A total of 19 OCPs were measured: four hexachlorocyclohexanes (α-hexachlorocyclohexane, β-hexachlorocyclohexane, γ-hexachlorocyclohexane, δ-hexachlorocyclohexane), six DDTs and metabolites (*p,p'*-dichlorodiphenyltrichloroethane (*p,p'*-DDT), *p,p'*-dichlorodiphenyldichloroethylene (*p,p'*-DDE), *p,p'*-dichlorodiphenyldichloroethane, *o,p'*-dichlorodiphenyldichloroethylene, *o,p'*-dichlorodiphenyldichloroethane, *o,p'*-dichlorodiphenyltrichloroethane), five chlordanes (*trans*-chlordane, *cis*-chlordane, oxychlordane, *trans*-nonachlor, *cis*-nonachlor), two heptachlors (heptachlor, hepatachlor epoxide), hexachlorobenzene, and mirex. Among them, we selected four OCPs for which at least 75% of the study participants had concentrations more than the limit of detection (LOD). LOD was defined as three times the signal to noise ratio. Participants with concentrations of OCPs below the LOD were given values of LOD divided by three. We used both wet-weight concentrations adjusted for serum cholesterol and triglyceride and lipid-standardized concentrations of OCPs by dividing wet-weight concentrations of OCPs by total lipids. Total lipids were calculated using the short formula: total lipids (mg/dL) = 2.27 × total cholesterol + triglycerides + 62.3. As both results showed similar associations, we presented the results based on lipid-standardized concentrations.

Statistical analysis

We examined the associations of serum concentrations of four individual compounds and the summary measure of OCPs with immunosenescence indicators of T lymphocytes (the frequencies of $CD4^+CD57^+$ or $CD4^+CD28^-$ cells among the $CD4^+$ T lymphocyte population, and $CD8^+CD57^+$ or $CD8^+CD28^-$ cells among the $CD8^+$ T lymphocyte population) using general linear models. Serum concentrations of each compound and summary measures of OCPs were divided into quartiles.

In human studies, analyses focusing on summary measure of OCPs are more reasonable than those on individual compounds because the serum concentrations of individual compounds are highly correlated in general populations (correlation coefficients among four compounds in this study: +0.27 to +0.70). In this situation, any interpretation focusing on individual compounds would be misleading. Among several methods of estimating the summary measures of POPs (6),

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we added the rank orders of individual compounds belonging to each subclass, which enable equal contributions from all the constituent compounds. This method can be preferred when the exact biological mechanism linking OCPs and immunosenescence is not known (6). Absolute concentration-based summary measures are problematic because they are mainly determined by *p,p'*-DDE due to much higher absolute concentrations than other compounds.

Important confounders considered in these analyses were age (continuous, years) and CMV IgG titer (continuous) because both of these are well-known risk factors for immunosenescence of T lymphocytes. We also considered sex, BMI, smoking status (never, ex, current), current drinking status (no, yes), and physical activity using the Korean version of International Physical Activity Questionnaire (www.ipaq.ki.se; low, moderate, and high) as possible confounders. However, as they were not confounders from the viewpoint of the definition of confounders and the adjustment for these variables did not materially change the results, they were not included in the final models.

In addition to statistical adjustment, the associations between OCPs and immunosenescence indicators were evaluated after being stratified by three categories of age (<40 years, 40–49 years, and ≥50 years) or tertile of CMV IgG titer. In stratified analyses, we presented results based on tertile of OCPs to increase statistical stability even though the patterns of results were similar between tertiles and quartiles of OCPs. Additionally, we redefined tertiles of OCPs using strata-specific cutoff points to balance the number of participants in each stratum. Furthermore, we presented results focusing on the comparison of the strength of association among age, CMV IgG titer, and OCPs. All data were analyzed using SAS version 9.1 (SAS Institute Inc.).

Results

Table 1 shows the general characteristics of study participants. Among 95 participants, 26% of the study participants were males. The mean age of the study participants was 44.8, ranging from 30 to 64 years, and the mean BMI was 24.4 kg/m^2. Current smokers, current drinkers, and persons with low physical activity were 15.8%, 53.7%, and 15.8%, respectively. The mean titer of CMV IgG was 10.6 and the range was 1.9 to 31.1. As the cut-off value for seropositivity of CMV IgG is 1.1, all participants yielded positive results for CMV IgG.

Table 1. General characteristics of study participants ($N = 95$)

Characteristics	
Mean ± SD	
Age (years)	44.8 ± 9.2
BMI (kg/m^2)	24.4 ± 3.7
Systolic blood pressure (mm Hg)	128.4 ± 17.9
Diastolic blood pressure (mm Hg)	78.8 ± 12.0
Fasting glucose (mg/dL)	91.2 ± 11.4
Total cholesterol (mg/dL)	186.6 ± 31.8
Triglycerides (mg/dL)	119.1 ± 71.1
HDL-cholesterol (mg/dL)	52.3 ± 12.3
CMV IgG titer	10.6 ± 4.6
Proportion (%)	
Men	27.4%
BMI ≥25 kg/m^2	40.0%
Current cigarette smoker	15.8%
Current alcohol drinker	53.7%
Low physical activity	15.8%
CMV IgG positive	100%

Table 2 shows the associations between serum concentrations of OCPs and the frequencies of $CD57^+$ and $CD28^-$ T lymphocytes among the $CD8^+$ and $CD4^+$ T-cell populations. Across the quartiles of the summary measure of OCPs, adjusted mean percentages of $CD8^+CD57^+$ and $CD8^+CD28^-$ T lymphocytes in the $CD8^+$ T lymphocyte population were 23.1, 27.9, 31.5, and 38.7 ($P_{trend} < 0.01$) and 24.8, 27.7, 28.9, and 35.4 ($P_{trend} < 0.01$). The relationship remained after adjustment for age and CMV IgG titer.

Among four OCPs, β-hexachlorocyclohexane showed the strongest positive associations with the percentages of senescent $CD8^+$ T lymphocytes in the $CD8^+$ T lymphocyte population. According to quartiles of serum concentrations of β-hexachlorocyclohexane, the adjusted mean percentages of $CD8^+CD57^+$ and $CD8^+CD28^-$ T lymphocytes in the $CD8^+$ T lymphocyte population were 24.8, 24.0, 34.5, and 37.9 ($P_{trend} < 0.01$) and 24.1, 23.0, 32.3, and 37.0 ($P_{trend} < 0.01$), respectively. Other OCPs, *p,p'*-DDT, *p,p'*-DDE, and *trans*-nonachlor, also showed positive associations with $CD8^+CD57^+$ T lymphocytes, but not $CD8^+CD28^-$ T lymphocytes ($P_{trend} = 0.04$ for *p,p'*-DDT; $P_{trend} = 0.02$ for *p,p'*-DDE; $P_{trend} < 0.01$ for *trans*-nonachlor). However, when age and CMV IgG titer were adjusted in the analyses, only *trans*-nonachlor was still statistically significant ($P_{trend} = 0.03$).

Contrary to $CD8^+CD57^+$ and $CD8^+CD28^-$ T lymphocytes, $CD4^+CD57^+$ and $CD4^+CD28^-$ T lymphocytes were not clearly related to OCPs (Table 2). Only *p,p'*-DDE showed an inverse association with the percentage of $CD4^+CD57^+$ T lymphocytes in the $CD4^+$ T lymphocyte population, but not that of $CD4^+CD28^-$ T lymphocytes. According to the quartiles of serum concentrations of *p,p'*-DDE, adjusted means were 5.5, 2.6, 2.6, and 2.3 ($P_{trend} = 0.02$).

In the stratified analyses by age (<40, 40–49, and ≥50 years), the association between OCPs and senescent $CD8^+$ T lymphocytes was remarkable among the youngest participants, aged less than 40 years (Fig. 1). However, the association became weaker with aging, and there was little relation among participants age greater than 50 years. In the stratified analyses by tertile of CMV IgG titer, the positive associations of OCPs with both $CD8^+CD57^+$ and $CD8^+CD28^-$ T lymphocytes became stronger with lower CMV IgG titers (Fig. 2). When we compared the strength of association among OCPs (quartile), CMV IgG titer (quartile), and age (quartile), which were included in one model, OCPs showed the strongest associations with both $CD8^+CD57^+$ and $CD8^+CD28^-$ T lymphocytes (Fig. 3).

Discussion

In this study, we demonstrated that background exposure to OCPs was positively related to the percentage of CD8+ T lymphocytes exhibiting cell surface characteristics that have been associated with senescence in the general population, and this relationship was stronger than those for known risk factors of immunosenescence, such as age or CMV IgG titer. Importantly, this relationship was more prominent among participants with lower risks based on known risk factors of immunosenescence, such as a young age or low CMG IgG titer. Notably, younger

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Table 2. Adjusted means of percentages of immunosenescence markers in $CD8^+$ or $CD4^+$ T lymphocytes according to quartile of summary measure of OCPs and serum concentrations of four individual OCPs ($N = 95$)

		Q1 ($n = 23$)	Q2 ($n = 24$)	Q3 ($n = 24$)	Q4 ($n = 24$)	P_{trend}	β (95% CI)
Summary measure of OCPs							
Summary score		**79**	**162**	**228**	**305**		
$CD8^+CD57^+$	Model 1	23.1	27.9	31.5	38.7	<0.01	+5.77 (2.92–8.64)
	Model 2	23.9	27.6	31.0	38.7	<0.01	+5.48 (1.95–9.01)
$CD8^+CD28^-$	Model 1	24.8	27.7	28.9	35.4	0.01	+4.11 (1.20–7.02)
	Model 2	25.6	27.3	28.0	35.5	0.02	+3.88 (0.32–7.44)
$CD4^+CD57^+$	Model 1	5.4	5.7	5.7	6.1	0.54	+0.23 (−0.53–0.99)
	Model 2	5.5	5.6	5.7	6.1	0.64	+0.16 (−0.78–1.11)
$CD4^+CD28^-$	Model 1	4.6	3.3	2.5	2.5	0.05	−0.64 (−1.44–0.16)
	Model 2	4.7	3.1	2.5	2.7	0.12	−0.58 (−1.56–0.40)
β-Hexachlorocyclohexane							
Median, ng/g lipid		2.4	4.8	7.0	10.7		
$CD8^+CD57^+$	Model 1	24.0	23.8	34.5	39.0	<0.01	+6.24 (3.42–9.06)
	Model 2	24.8	24.0	34.5	37.9	<0.01	+5.37 (2.33–8.41)
$CD8^+CD28^-$	Model 1	24.0	22.8	32.0	37.6	<0.01	+5.51 (2.70–8.32)
	Model 2	24.1	23.0	32.3	37.0	<0.01	+4.89 (1.87–7.91)
$CD4^+CD57^+$	Model 1	5.4	5.2	6.2	6.1	0.35	+0.50 (−0.26–1.26)
	Model 2	5.4	5.2	6.3	6.0	0.44	+0.42 (−0.40∼1.25)
$CD4^+CD28^-$	Model 1	3.8	2.8	3.5	2.8	0.55	−0.13 (−0.95∼0.68)
	Model 2	3.5	2.8	3.7	2.9	0.84	−0.07 (−0.93–0.79)
p,p′-DDT							
Median, ng/g lipid		1.7	3.8	5.1	8.4		
$CD8^+CD57^+$	Model 1	25.2	27.5	36.8	31.8	0.04	+3.86 (0.86–6.85)
	Model 2	27.0	27.8	35.9	30.6	0.27	+2.65 (−0.68–5.98)
$CD8^+CD28^-$	Model 1	25.2	27.4	35.6	28.2	0.21	+2.81 (−0.17–5.78)
	Model 2	26.8	27.3	34.9	27.5	0.61	+1.93 (−1.37–5.22)
$CD4^+CD57^+$	Model 1	5.0	6.1	6.2	5.5	0.65	+0.11 (−0.66–0.87)
	Model 2	5.2	6.1	6.1	5.5	0.80	+0.00 (−0.86–0.86)
$CD4^+CD28^-$	Model 1	2.8	4.5	3.4	2.2	0.41	−0.37 (−1.18–0.44)
	Model 2	2.7	4.2	3.5	2.4	0.75	−0.22 (−1.12–0.68)
p,p′-DDE							
Median, ng/g lipid		19.9	45.9	70.0	127		
$CD8^+CD57^+$	Model 1	26.9	24.3	36.4	33.8	0.02	+3.14 (0.12–6.17)
	Model 2	28.4	24.9	35.9	32.2	0.16	+1.76 (−1.55–5.07)
$CD8^+CD28^-$	Model 1	28.5	22.7	34.2	31.2	0.14	+3.23 (0.27–6.19)
	Model 2	29.5	23.3	34.0	29.8	0.47	+2.43 (−0.81–5.67)
$CD4^+CD57^+$	Model 1	6.3	5.4	5.8	5.5	0.54	−0.22 (−0.99–0.54)
	Model 2	6.6	5.5	5.7	5.2	0.32	−0.41 (−1.26–0.44)
$CD4^+CD28^-$	Model 1	5.5	2.5	2.6	2.4	0.01	−0.80 (−1.59–0.00)
	Model 2	5.5	2.6	2.6	2.3	0.02	−0.77 (−1.65–0.10)
Trans-nonachlor							
Median, ng/g lipid		2.4	4.8	7.0	10.7		
$CD8^+CD57^+$	Model 1	21.9	31.5	32.8	34.9	<0.01	+4.05 (1.07–7.03)
	Model 2	23.2	31.9	32.3	33.7	0.03	+2.97 (−0.26–6.20)
$CD8^+CD28^-$	Model 1	23.0	31.3	30.5	31.5	0.07	+2.60 (−0.38–5.58)
	Model 2	24.2	31.6	30.0	30.6	0.23	+1.74 (−1.48–4.96)
$CD4^+CD57^+$	Model 1	4.7	6.0	6.6	5.5	0.37	+0.15 (−0.62–0.91)
	Model 2	4.7	6.0	6.5	5.6	0.44	+0.07 (−0.77–0.91)
$CD4^+CD28^-$	Model 1	2.8	4.8	2.9	2.3	0.32	−0.50 (−1.31–0.30)
	Model 2	2.7	4.7	2.9	2.6	0.62	−0.39 (−1.27–0.48)

Model 1: Unadjusted.
Model 2: Adjusted for age (continuous) and CMV IgG titer (continuous).
β (95% CI): beta-coefficient per 1 SD unit increase (95% confidence interval).

participants with OCPs in the highest tertile showed the highest percentage of $CD8^+CD57^+$ T lymphocytes and the lowest variation; this percentage was even higher than that of older participants.

Among the four indicators of immunosenescence markers evaluated in this study, $CD8^+CD57^+$ T lymphocytes showed the clearest association with serum concentrations of OCPs. In fact, shifts in $CD8^+$ T-cell subsets are hallmarks of immunosenescence in humans and are observed in ageing and in conditions of chronic immune stimulation, such as cancer (11). Repetitive antigenic stimulation leads to the gradual accumulation of late-differentiated, antigen-specific, oligoclonal T cells, particularly within the $CD8^+$ T-cell compartment (14).

It was once thought that CD28 and CD57 expression was mutually exclusive in human T lymphocytes (15), and repetitive antigenic stimulation gradually converted early-differentiated memory $CD28^+CD57^-$ T cells into end-stage-differentiated $CD28^-CD57^+$ T cells. However, as $CD8^+CD57^+$ T lymphocytes have undergone the same number of cell divisions irrespective of CD28 and have the shortest telomeres

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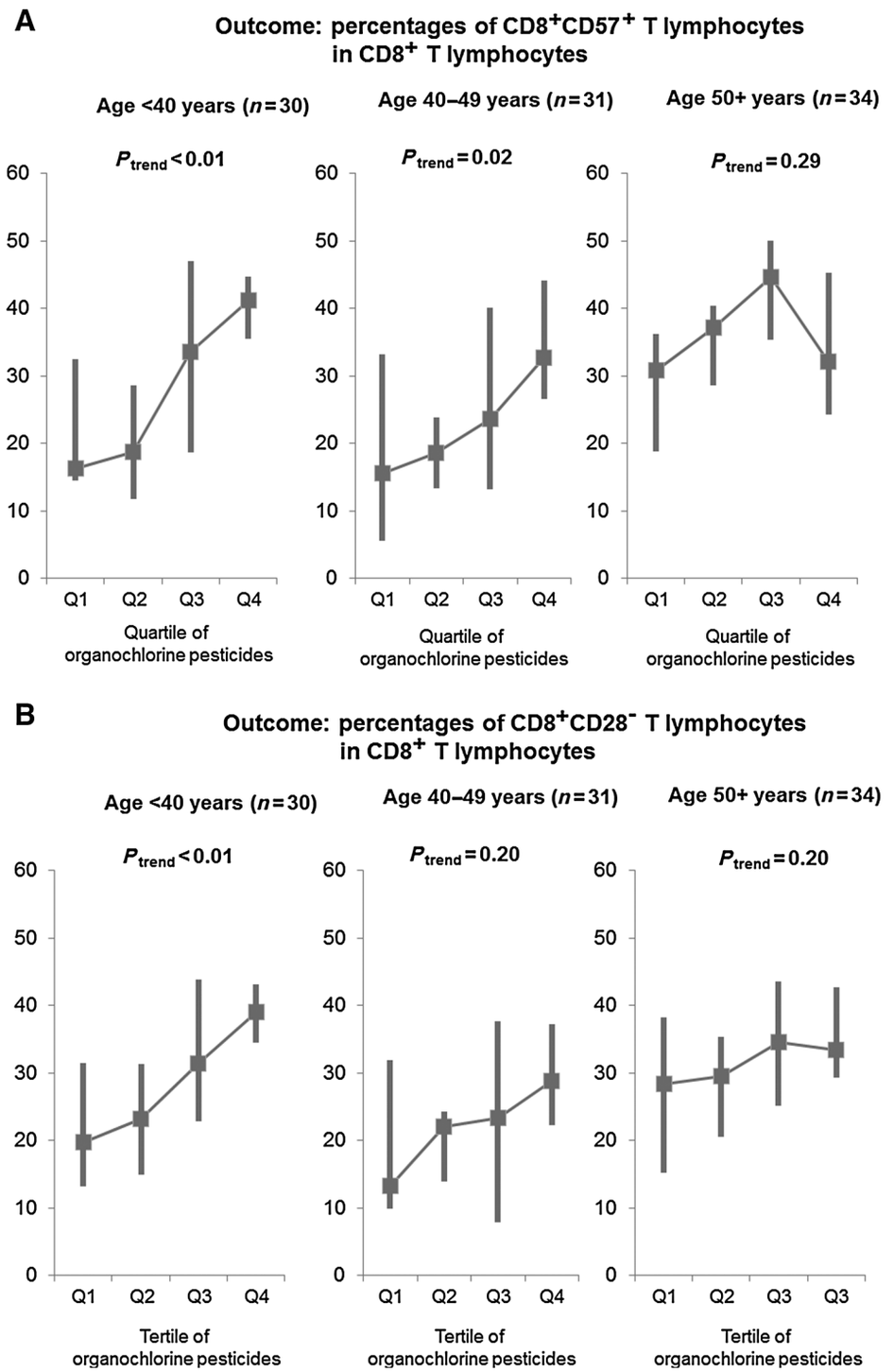

Figure 1.
Association between summary measure of OCPs and percentages of immunosenescence markers $CD8^+CD57^+$ (**A**) and $CD8^+CD28^-$ (**B**) T lymphocytes in $CD8^+$ T lymphocytes stratified by age. Tertiles of OCPs were redefined by strata-specific cutoff points to increase statistical stability. Median values of tertiles of OCPs in each stratum were 62, 107, and 168 among participants age <40 years; 133, 190, and 285 among participants ages 40–49 years; and 168, 255, and 326 among participants age ≥50 years. Box: median; low margin: 25% value; high margin: 75% value.

(13), some researchers have suggested that expression of CD57 alone most accurately predicts replicative senescence of $CD8^+$ T cells (16). Supporting this idea, in this study, serum concentrations of OCPs were more strongly associated with $CD8^+CD57^+$ T cells rather than $CD8^+CD28^-$ T cells.

As $CD8^+CD57^+$ and $CD8^+CD28^-$ T lymphocytes are highly oligoclonal, their accumulation reduces the overall diversity of the $CD8^+$ T-cell compartment in terms of antigen specificity (14). This is associated with incompetent immune function, that is, reduced overall immune response to pathogens, vaccines, and tumor cells. In fact, the $CD8^+CD57^+$ T-cell population is related to various common clinical conditions, especially among the elderly (1, 3, 11). However, the strong association between OCPs and the $CD8^+CD57^+$ T-cell population

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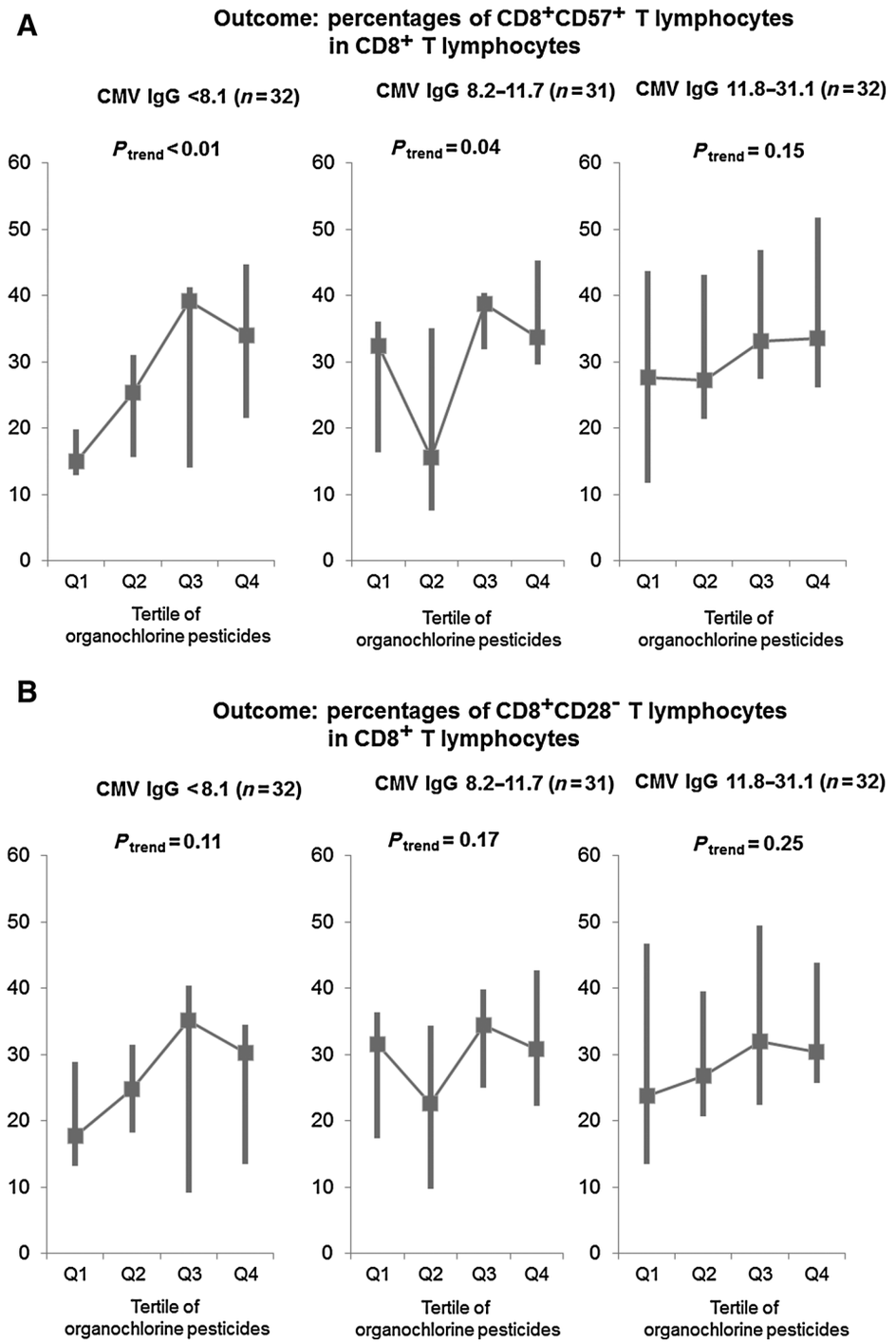

Figure 2.
Association between summary measure of OCPs and percentages of immunosenescence markers $CD8^+CD57^+$ (**A**) and $CD8^+CD28^-$ (**B**) T lymphocytes in $CD8^+$ T lymphocytes stratified by CMV IgG titer. Tertiles of OCPs were redefined by strata-specific cutoff points to increase statistical stability. Median values of tertiles of OCPs in each stratum were 86, 168, and 272 among participants with CMV IgG <8.1; 95, 200, and 289 among participants with CMV IgG 8.2–11.7; and 104, 172, and 301 among participants with CMV IgG 11.8–31.1. Box: median; low margin: 25% value; high margin: 75% value.

in young adults suggests that chronic exposure to persistent chemicals, such as OCPs, may contribute to the increased risk of these clinical conditions, even among young adults.

At present, aging is the most well-established risk factor of immunosenescence (17). Aging is associated with progressive accumulation of $CD8^+CD57^+$ and $CD8^+CD28^-$ T lymphocytes, presumably as a result of lifetime exposure to common persistent antigens, such as CMV (17). Thus, young adults have substantial accumulations of 20–30% of $CD8^+CD57^+$ and $CD8^+CD28^-$ (18), and elders have above approximately 50–60% of $CD8^+$ T cells with CD57 expression (19). In this study, however, age was not clearly associated with immunosenescence among all study participants ages 30 to 64 years. One reason for this may be that this study did not include older participants, such as octogenarians or nonagenarians, who were the main targets in previous studies (20).

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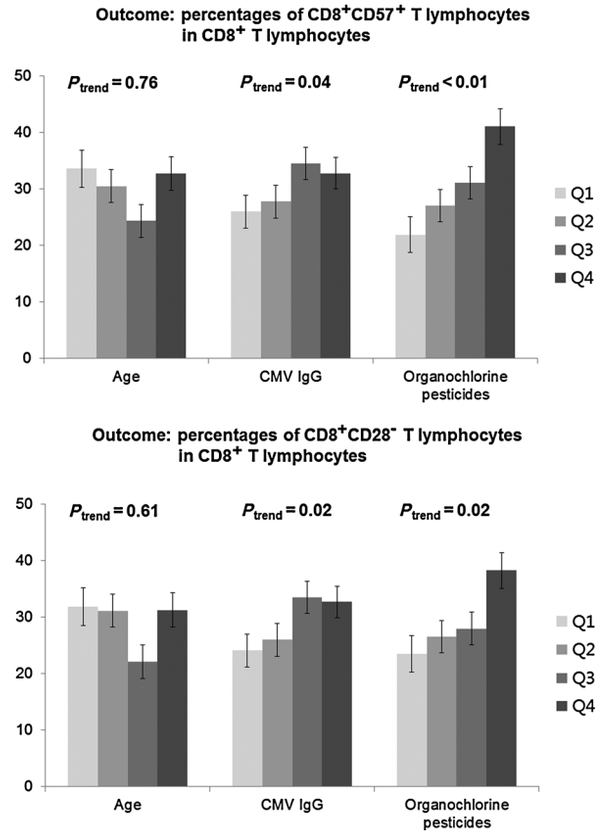

Figure 3.

Comparison of strength of associations with percentages of immunosenescence markers in $CD8^+$ T lymphocytes among age, CMV IgG, and OCPs.

Also, it is well known that the increase of the $CD8^+CD57^+$ and $CD8^+CD28^-$ T-cell lymphocytes is observed in individuals with chronic viral infections (21). Chronic latent CMV infection, especially, markedly alters the numbers and proportions of peripheral immune cells (4). In this study, the known relationships between CMV IgG and immunosenescence markers of $CD8^+$ T lymphocytes were also observed. However, OCPs showed stronger associations with $CD8^+$ T lymphocyte senescence than CMV IgG titer. Some researchers have suggested that viral infections, rather than age per se, are attributed to the expansion of the $CD8^+CD57^+$ and $CD8^+CD28^-$ T lymphocytes with aging (22). Interestingly, the strong positive association between age and serum concentrations of persistent organic pollutants, such as OCPs, is well known (23). Thus, aging-related immunosenescence needs to be reevaluated from the viewpoint of persistent chemical contamination as well as chronic viral infections.

OCPs have been widely used since after World War II (6). Although their use was banned several decades ago in most countries, the exposure to very low doses of these chemicals continues in general populations (6). The most important source of external exposure is fatty animal food such as fish, meat, and milk because these lipophilic chemicals persist in the environment and undergo bioaccumulation and biomagnification through the food chain (9). Furthermore, adipose tissue plays an important role as an internal source of exposure. When OCPs enter into the body from the environment, they are primarily stored in adipose tissue and continuously released into the circulation through controlled and uncontrolled lipolysis (7). Thus, even though there is no external exposure source to these chemicals, OCPs are still detected in the body. Finally, it is important to note that the exposure to OCPs starts from the fetal period *in utero*, because OCPs are transferred from mother to fetus across the placenta (24). Also, breastfeeding is a major source of exposure because breastmilk is a typical fatty animal food contaminated with OCPs (25). All these features on exposure sources of OCPs explain why young subjects of the current general population have substantial levels of OCPs that were banned before their birth.

Therefore, contemporary general populations would be seen as persons who have been exposed to these chemicals throughout their lifetime. This suggests that chronic exposure to very low doses of these chemicals can affect the immune system. Although direct immunotoxicity of high-dose OCPs has been reported in earlier *in vitro* and *in vivo* experimental studies (26, 27), replicative senescence of T lymphocytes due to low-dose chronic exposure to OCPs may have different molecular mechanisms from direct immunotoxicity of high-dose OCPs, which should be investigated in future experimental studies.

However, it is important to note that any estimate of serum concentrations of OCPs in humans should be interpreted as the result of strong lipophilic chemical mixtures that move together with OCPs in the environment and our body, irrespective of whether they are measured (28). In fact, chronic exposure to low-dose OCPs and other strongly correlated lipophilic chemicals has recently been linked to a variety of chronic diseases, including type 2 diabetes, cardiovascular diseases, rheumatoid arthritis, and cancer, in human studies in which the mechanisms are unclear (6, 29–31). Immunosenescence may be one possible mechanism.

Although immunosenescence is mechanistically linked to carcinogenesis, there is no prospective study to evaluate the relationship of immunosenescence and the risk of developing cancer in the general population. This is understandable because immunosenescence can be assessed only when it is included as part of the study design at the planning stage because of the requirement of a large amount of fresh blood. However, poor survival among cancer patients with high proportions of immunosenescence markers has been reported (32, 33).

Our study has several limitations. First, the cross-sectional study design may not permit causal inference. However, as the large amount of fresh blood (20 ml) is required for immunophenotyping, this hypothesis cannot be easily tested in most established cohorts only with stored serums and DNA. Also, reverse causality may not be the plausible explanation for the current findings because it is difficult to assume that the deterioration of immune system could increase serum concentrations of OCPs with very long half-lives of several years to decades. Second, the status of CMV infection was indirectly estimated by the CMV IgG titer, not the direct quantitation of CMV latent dose or viral titer measured by CMV genome copies. Third, there could be immunosenescence-related lifestyle factors (34, 35), which were not properly considered in the current study. Even though regular exercise has been reported to have an anti-immunosenescence effect in intervention studies (36), it would

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be difficult to detect this association in the current observational study. Fourth, some findings could be the result of chance due to the small sample size.

Conclusion

In conclusion, we have demonstrated that chronic exposure to OCPs may be an important driving force of the replicative senescence of T lymphocytes. In particular, the strong association was observed among subjects without known risk factors of T-cell immunosenescence. Although our findings need to be replicated in other populations, T cell immunosenescence may be one possible mechanism linking low dose OCPs and many chronic diseases.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Authors' Contributions

Conception and design: H.T. Yu, E.-C. Shin, D.-H. Lee
Development of methodology: H.T. Yu, H.-C. Kim
Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): H.T. Yu, S.-A. Kim, S.-H. Hong, Y.-R. Yoon, D.-J. Kim, H.-C. Kim, H.-B. Moon
Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): H.T. Yu, S.-A. Kim, H.-B. Moon, E.-C. Shin
Writing, review, and/or revision of the manuscript: D.-H. Ryu, H.T. Yu, Y.-M. Lee, D.-J. Kim, H.-C. Kim, H.-B. Moon, E.-C. Shin, D.-H. Lee
Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): D.-H. Ryu, H.T. Yu, S.-A. Kim, S.-H. Hong, D.-J. Kim, D.-H. Lee
Study supervision: H.T. Yu, E.-C. Shin, D.-H. Lee

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