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ORIGINAL ARTICLE

Relationships of climatic data to immune and hematologic variables in normal human

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Abstract

OBJECTIVES: The purpose of this study was to examine the relationships between climatic and immune or hematologic variables in the peripheral blood of normal human. METHODS: Twenty-six normal volunteers gave blood samples monthly during one calendar year for flow cytometric assays of peripheral blood mononuclear cells (PBMC) and assays of red blood cell (RBC)- and platelet-related variables. Time relationships between the weather and immune or hematologic variables were investigated by means of multiple regression and bivariate cosinor analyses. RESULTS: Highly significant relationships were found among number and percentage of neutrophils, lymphocytes, $CD4^+$, $CD8^+$, $CD25^+$ (interleukin-2-receptor bearing lymphocytes), $CD20^+$ B lymphocytes, number of platelets and RBC, hemoglobin (Hb), mean corpuscular (MC) volume, MC Hb, MC Hb concentration, mean platelet volume or plasma fibrinogen levels and ambient temperature, sunlight duration, air pressure, wind speed, relative humidity, and rainfall duration/day. An important part of the variability in the immune and hematologic variables could be explained by the composite effects of contemporaneous and lagged climatic variables. Common seasonal rhythms were detected in the time series of the above immune/hematologic and sun insolation variables, such as ambient temperature. CONCLUSIONS: The results suggest that i) short-term fluctuations in atmospheric activity modulate immune and hematologic features in the peripheral blood of normal human; and ii) the seasonal rhythms observed in immune/hematologic variables may be entrained by the seasonal rhythms in ambient temperature.

Introduction

In humans, significant seasonal and daily rhythms have been observed at several levels of the immune and hematopoietic systems. Significant circadian rhythms are now well established in function, proliferation and number of peripheral blood immune cells [1–3], platelets and red blood cells (RBC) [2-4]. Comparatively fewer studies have focused on the annual variations in the above variables probably due to the practical limitation of obtaining multiple blood samples during a longer period of time. Nevertheless, some research groups have reported seasonal differences in immune and hematopoietic variables, such as in number of total lymphocytes, $CD4^+$ T (T helper-inducer), $CD8^+$ T (T suppressor-cytotoxic) [5, 6] and B lymphocytes [7]. Other groups found seasonal differences in the number of RBC, hematocrit (Ht) and hemoglobin (Hb) in elderly subjects [8].

However, previous research on seasonal variation in immune/hematologic variables was often limited by the cross-sectional design of the studies, the smaller number of healthy subjects and of time points for blood samplings in transversal or prospective studies, and by the use of statistical tests, which was often less than optimal (e.g. simple t-tests or analyses of variance applied to detect differences across the seasons; single cosinor). In a larger number of healthy controls (n=26) who had monthly blood samplings during one calendar year, our laboratory has quantified the seasonal variation in various immune and hematologic variables by means of spectral and cosinor analyses of single and grouped time series [9, 10]. Highly significant seasonal rhythms (i.e. annual, semiannual, tetramensual and/or trimensual), which were expressed as a group phenomenon, were found in number or percentage of peripheral blood neutrophils, lymphocytes, monocytes, $CD3^+$ T, $CD4^+$ T, $CD8^+$ T, $CD25^+$ T and $CD20^+$ B lymphocytes, number of RBC and platelets, Hb, Ht, mean corpuscular volume (MCV), MC Hb (MCH), MCH concentration (MCHC), RBC distribution width (RDW), mean platelet volume (MPV), and plasma fibrinogen [9, 10].

There is limited information on the origin of these yearly rhythms in the above immune and hematologic variables. It is thought that circadian and seasonal rhythms in biological variables in human, including those in the immune system, are endogenously (i.e. genetically) determined and/or entrained or modulated by environmental "zeitgebers" such as climatic factors [2, 11, 12]. There are only a few studies reporting that climatic variables, such as ambient temperature, may induce changes in lymphocyte and monocyte counts in the rodent [13–15]. Lowering of ambient or body temperature may also be accompanied by an increase in number of RBC and platelets [16].

The aims of the present study were to examine i) whether immune and hematologic variables are related to short-term fluctuations in atmospheric activity; and ii) whether there are synchronized seasonal rhythms in the above immune/hematologic variables and sun insolation data, such as ambient temperature.

Methods

Subjects

Inclusion criteria for subjects to participate in this study were: **i)** genuine Caucasian volunteers living in Antwerp (geographical coordinates: 51.2°N and 4.5°E), Belgium, who were free of any psychiatric and medical illnesses (e.g. immune or endocrine illness) and drugs (including the pill). Past history of psychiatric and personality disorders was checked by means of the Semi-Structured Clinical Interview of the DSM-III-R [17, 18]. Subjects with drug (alcohol, and any other drugs of dependence) use or abuse were not included. **ii)** A stable, settled lifestyle. Therefore, subjects regularly taking international flights and subjects who travel a lot, such as commercial travellers, were not included in this study. **iii)** Women of childbearing potency were only included if they were willing to ascert that they would not become pregnant during this study period. **iv)** Subjects were not allowed to spend more than one week in another country and were not allowed to travel outside an area of more than 250 km from the town of Antwerp.

Exclusion criteria were: **i)** abnormal chemical and hematologic blood tests the first study month, such as complete blood count, blood urea, nitrogen, electrolytes, liver enzymes (SGPT, SGOT, γGT), hemoglobin, hematocrit. **ii)** The occurrence of a new medical illness during the study period, except a common cold or angina, or any use of new medical drugs, except occasional use of an over-the-counter drug such as aspirin (prohibited for at least two days before blood sampling). In case of a common cold, blood samplings were deferred until clinical remission. **iii)** The occurrence of important negative life events during the study period. **iv)** Tobacco use of more than five cigarettes a day.

Finally, 26 normal volunteers (13 men, 13 women; mean age=38.7; SD=13.4 years; range: 23–69 years) were selected to participate in this study. Volunteers had the socio-economical status of the middle-class Belgian population and a mean, net monthly income between $1,500 and $2,500; they were urbanized persons with a comparable rest-activity schedule.

The study period extended from December 11, 1991 until December 25, 1992. Sixteen subjects had their first blood sampling in December 1991; the others started in January 1992.

Procedures

The preparation of the subjects prior to blood collection was carefully controlled and blood collections were performed in standardized conditions in order to minimize sources of preanalytical variability. The same two MDs carried out all blood samplings throughout the study period on the same subjects. Subjects had 12 consecutive monthly blood samplings. Blood samplings in premenopausal females were carried out 5–10 days after the first day of the menstrual cycle. After a vacation in an area other than the province of Antwerp, blood was sampled at least seven days after returning to Antwerp-town. There were 59 days on which blood was sampled. Dates of blood samplings were randomly distributed during the study span. Blood samples were taken in a thermally controlled indoor environment and all subjects were allowed to acclimate to the room for 60 minutes prior to the blood collections. They wore street clothes during the study. The temperature in the testing room was maintained at 21.0±1.5°C.

An intravenous cannula was inserted at 8:00 a.m. (±30 min) in the antecubital vein of the subjects and 65 mL of blood was drawn from the fasting subjects. Flow cytometry for the determination of percentage and number of PBMC was performed on fresh blood as previously described by us [9]. Labelled monoclonal antibodies against CD4, CD8, CD3 and CD25 were purchased from Dakopatts (Denmark, Copenhagen) and CD20 from Becton Dickinson (Mountain View, CA, USA). We have used the following antibodies: $CD3^+$ (pan T cells), $CD4^+$ (T helper-inducer cells), $CD8^+$ (T suppressor-cytotoxic cells), $CD25^+$ (interleukin-2-receptor bearing lymphocytes), and $CD20^+$ (B lymphocytes). All measurements of the different leukocyte subsets in the present study were obtained using the same lot of monoclonal antibodies. Quality control performed through calibration with Quick Call (Becton Dickinson, Mountain View, CA, USA), showed that highly reproducible results were obtained in our laboratory during the course of this study. The results are expressed as the percentage (%) or absolute number (AN) of leukocytes. White blood cell count, leukocyte differentiation (i.e. lymphocytes), RBC- and platelet-related parameters were determined on fresh blood by means of a Coulter STKS fully automated total blood cell counter. Hb was determined by means of the cyanmethemoglobin method using a dilution of blood in a solution containing potassium cyanide and ferricyanide. Fibrinogen was determined by means of fixed time kinetic nephelometry for immunoprecipitation using the Behring Nephelometer (Behringwerke AG, Marburg/Lahn, Germany). The analytical interassay coefficients of variation are for number of leukocytes: 1.3%; lymphocytes: 2.5%; RBC: 0.4%; Hb: 0.6%; Ht: 0.8%; MCV: 0.6%; MCH: 0.7%; MCMC: 1.0%; RDW: 1.4%; number of platelets: 1.7%; MPV: 1.4%; and fibrinogen: 5%.

Weather data for the vicinity were taken at the Royal Meteorological Station of Deurne which is situated in the eastern part of Antwerp City and comprised mean daily atmospheric pressure (hPa), air temperature (°C), relative humidity (%) and wind speed (km/h), together with minutes of sunlight and precipitation/day.

Statistics

Relationships between biological and weather variables were investigated by two techniques, i.e. bivariate cosinor and regression analyses. The biological data were correlated to the same day's meteorological data. Bivariate cosinor analyses were employed as previously described by us [19–22]. We examined the significance of annual rhythms as well as the harmonics, i.e. semi-annual (180 days), four-monthly (120 days), tri-mensual (90 days) and bi-monthly (60 days) rhythms. In order to eliminate the effects of interindividual variability in the analyses of the time series of immune/hematologic data, each of these data was normalized with reference to the yearly mean values in each of the 26 normal volunteers. The regression analyses were pooled over the 26 time series of the normal persons in order to minimize the interindividual variability in the immune/hematologic data. The time series of the immune/hematologic data were entered as dependent variables and the climatic data were entered as independent or explanatory variables. When evaluating the possible impact of the weather condition on plasma variables, it may be assumed that the latter may respond to a set of climatic variables which work in concert rather than individually (i.e. synergy) and that it takes a period of time for the climate to affect plasma constituents (i.e. the memory effect). Our laboratory has shown that the memory effects of climatic variables on plasma constituents and behavioral features in normal human often involve a time lag of one to four weeks [22–24]. To investigate possible synergistic and memory effects, a multiple regression model is employed whereby present together with lagged climatic data are entered as explanatory variables [21–23]. Time lags of 1–4 weeks of the climatic

variables are entered in multiple regression analyses as additional explanatory variables. In order to prevent multicollinearity, the authors have performed multiple regression analyses whereby the climatic variables are entered in a forward stepwise (step-up) automatic inclusion method (with an F to enter of p=0.05) with inspection of the determinant.

In order to show the time relationships between immune/hematologic variables and ambient temperature, we made a display of the cyclic signals in the raw values of both variables. The cyclic signals in the raw data reflect the total amount of variance in the raw data, which can be explained by the significant seasonal rhythms. For more explanation on these techniques, the reader is referred to some of our previous publications [20, 21, 24].

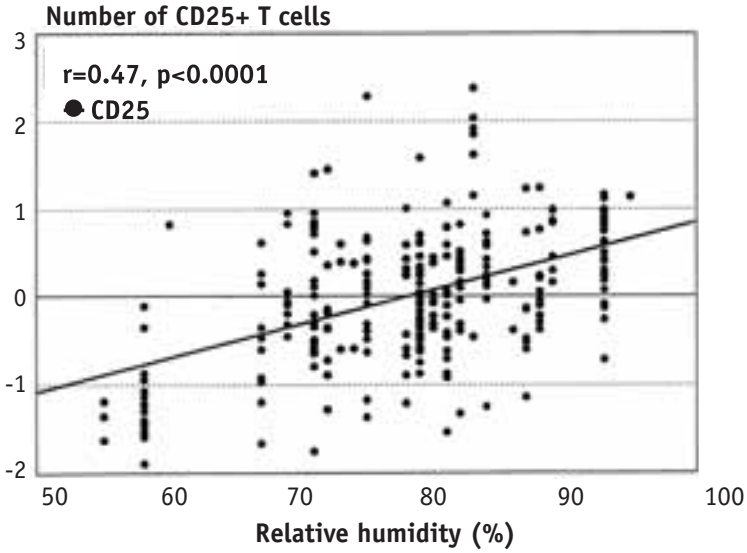

Fig. 1. Regression of number of $CD25^+$ T cells in the peripheral blood on relative humidity.

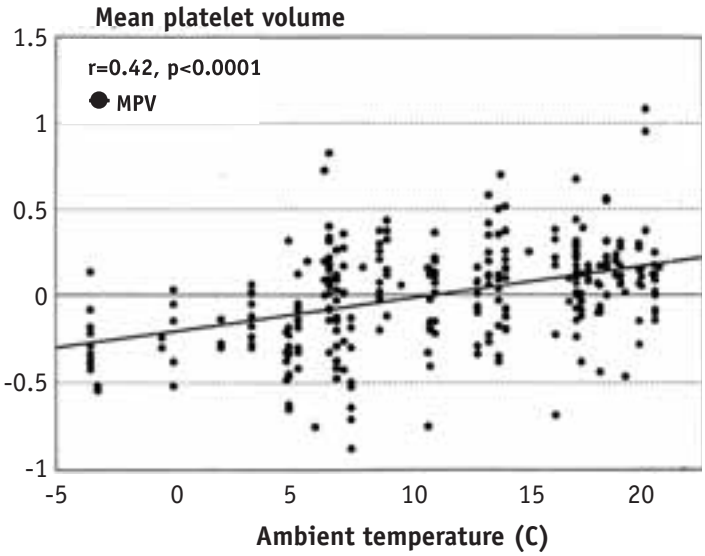

Fig. 2. Regression of mean platelet volume (MPV) on ambient temperature.

Results

Results of univariate regression analyses

The number of significant correlations in the correlation matrix between each of the immune variables and the 30 past and lagged climatic variables (at the p=0.05 level and pooled over the 26 time series of the subjects) were as follows: leukocytes 3; AN neutrophils 8; %neutrophils 11; AN monocytes 3; %monocytes 4; AN lymphocytes 9; %lymphocytes 13; AN $CD3^+$ 6; $\%CD3^+$ 19; AN $CD4^+$ 16; $\%CD4^+$ 10; AN $CD8^+$ 11; $\%CD8^+$ 12; AN $CD25^+$ 11; $\%CD25^+$ 15; AN $CD20^+$ 22; $\%CD20^+$ 18; and $CD4^+/CD8^+$ ratio 14. Figure 1, for example, shows the positive relationship between the number of $CD25^+$ cells in the peripheral blood and relative humidity.

The number of significant correlations in the correlation matrix between each of the hematologic variables and the 30 climatic variables (at the p=0.05 level) were as follows: number of RBC 11; Hb 3; Ht 5; MCV 16; MCH 15; MCHC 17; RDW 12; number of platelets 13; MPV 21; and fibrinogen 14. For example, Figure 2 shows the positive correlation between MPV and ambient temperature.

Results of multiple regression analyses

Table 1 shows the outcome of 17 multiple regression analyses with stepwise inclusion of the climatic data. The relevant climatic data are shown by means of the significance of their regression coefficients. For example, 17.4% of the variance in the percentage of neutrophils was explained by three climate variables, i.e. present and past air pressure and relative humidity. It is interesting to note that present and lagged climatic data are sometimes included in the same regression equation, e.g. air pressure in the case of percentage and number of neutrophils and lymphocytes. This indicates that a change in air pressure over the past few weeks is related to changes in these immune cells. Therefore, we have investigated the relationships between changes in air pressure (i.e. Δ air pressure = present values - past value at a lag of three weeks) and the percentage of those immune cells. Figures 3 and 4 show the significant correlations between Δ air pressure and percentage of neutrophils and lymphocytes, respectively.

Table 2 lists the results of multiple regression analyses (pooled over the 26 time series of the 26 subjects) with stepwise inclusion of cli-

Table 1. Results of multiple regression analyses of immune data on past and present climatic data

Dependent variable	Significant explanatory variables	Multiple regression analyses			
		R^2(%)	F-statistic	df	p-value
Leukocytes[1]	+W[§], +W4[§]	5.2	7.8	2 /289	0.008
% Neutrophils	+P3*, -P * I -H[§]	17.4	19.8	3/281	$<10^{-4}$
AN Neutrophils[1]	-T4*, +P3[$], -P[$], -R1[§] 0 +T2[§], +S, -P	19.2	8.9	7/262	$<10^{-4}$
% Lymphocytes	+P*, +T4[$], +H[$]; -P3[§] + +R3, +R1	22.7	13.6	6/278	$<10^{-4}$
AN Lymphocytes	-P3*, +P[§]	9.4	13.8	2/267	$<10^{-4}$
% Monocytes	-T2*, -SI*, -H1*, +T[$], -R4[$], +R2[§], -P2[§] +T4, +R	21.1	8.2	9/275	$<10^{-4}$
AN Monocytes	+R2[$], +P4[§], +R[§]	8.6	8.3	3/266	0.0001
% $CD3^+T$	-H3*, -R4[§], +W2[$], +R[$], -P2[§], -T3[§], -W[§]	21.7	9.5	8/275	$<10^{-4}$
% $CD4^+T$	-P*, -T*, +P1*, +W4*, +W2*, +T2[§],-H3[§]	25.4	13.4	7/277	$<10^{-4}$
AN $CD4^+T$	+P2*, +W4*, +H4*, +R3*, -H1[$], +T4	21.6	12.0	6/261	$<10^{-4}$
% $CD8^+T$	-T3*, -R4*, -H4*, +S*, -W3[$], +W2[$], -P2[§]	26.3	14.1	7/277	$<10^{-4}$
AN $CD8^+T$	+T*, -T3*, +P*, -R1[§], -R4, -P1, -S3	18.9	8.6	7/260	$<10^{-4}$
$CD4^+/CD8^+$ ratio[1]	+P2*, +W4*, +T2*, +H4*, -S2*, -P3*, -S*, -H1[$], +S4[§]	33.6	15.5	9/275	$<10^{-4}$
% $CD25^+T$[1]	-S4*, +H4[$],+H[$], +T2*, -W1[§], +P4[§], +P2	42.5	29.2	7/277	$<10^{-4}$
AN $CD25^+T$[1]	-S4*, +H*, +T2*, +P1[$], +H4[§], +R4[§],-W4, +P2	43.4	24.8	8/259	$<10^{-4}$
% $CD20^+$ B	-P1[$], +H4[$], +P2[$], -P3[§], -W2[§], -P4[§], +R3, -R	27.7	11.6	9/273	$<10^{-4}$
AN $CD20^+$ B	+H4*, +P2[$], -P1[$], -R1[$], -P3[$], +W4[$], +W3	28.0	14.3	7/258	$<10^{-4}$

Listed are the results of pooled multiple regression analyses (i.e. pooled over the 26 subjects) with the immune values in 26 normal controls as dependent variables and climatic data as explanatory variables. Present and lagged (1, 2, 3 and 4 weeks) values of air pressure (P), sunlight duration (S), wind speed (W), ambient temperature (T), rainfall duration (R), and relative humidity (H) are entered in pooled multiple regression analyses; 1, 2, 3 and 4 denote a time lag of 1, 2, 3 or 4 weeks, respectively.

[1] These immune variables were processed in logarithmic transformation.

* $p < 0.0001$; [$] $p < 0.001$; [§] $p < 0.01$; without index: $p < 0.05$.

matic data with an F to enter of p=0.05. The relevant explanatory climatic variables are listed by means of the significance of their regression coefficients.

Results of bivariate cosinor analyses

In order to determine whether there are common seasonal rhythms (i.e. annual, semi-annual, four-monthly, trimonthly and bimonthly rhythms) in the time series of the immune/hematologic data and ambient temperature or changes in ambient temperature the week prior to blood sampling, we have performed bivariate cosinor analyses. Ambient temperature or changes in ambient temperature were chosen as the relevant climate variables since our previous research has shown that i) the seasonal rhythms in ambient temperature are strongly synchronized with those in relative humidity and sunlight duration; and ii) there are highly significant seasonal rhythms in changes in ambient temperature, which are strongly related to human plasma analytes and behaviors [20, 21, 25].

By means of bivariate cosinor analyses we detected significant common rhythms in ambient temperature and the number of $CD25^+$ T cells (180 days: $F=12.6$, $p<0.01$; 90 days: $F=27.2$, $p<0.01$; 60 days: $F=9.1$, $p<0.01$), and (after rotation of the time series of ambient temperature by 180°) also with the $CD4^+/CD8^+$ ratio (120 days: $F=6.3$, $p<0.01$; 90 days: $F=4.4$, $p<0.05$), and number of $CD20^+$ B cells (180 days: $F=5.7$, $p<0.01$; 120 days: $F=5.1$, $p<0.01$). By means of bivariate cosinor analyses we detected significant common rhythms in the changes in ambient temperature and the $CD4^+/CD8^+$ ratio (120 days: $F=13.5$, $p<0.01$); number of $CD20^+$ B cells (120 days: $F=20.2$, $p<0.01$); and number of $CD20^+$ B cells (180 days: $F=6.3$, $p<0.01$; 120 days: $F=18.8$, $p<0.01$; 90 days: $F=18.4$, $p<0.01$; 60 days: $F=6.3$, $p<0.01$). Figure 5 shows the negative time relation-

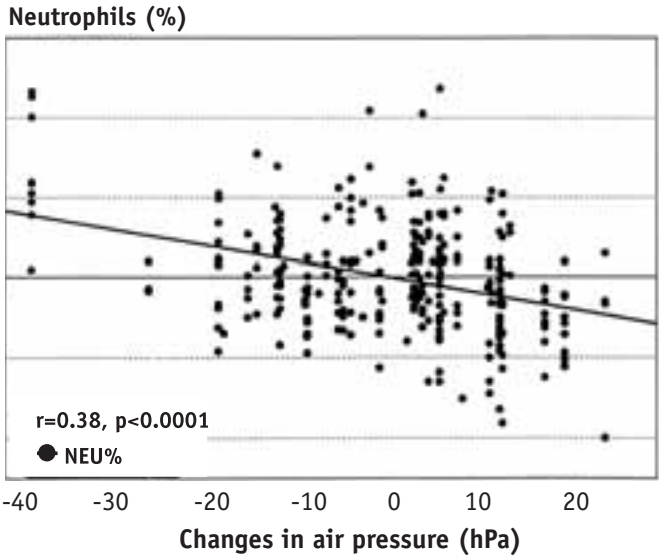

Fig. 3. Regression of percentage of neutrophils in the peripheral blood on changes in Δ air pressure during the last three weeks.

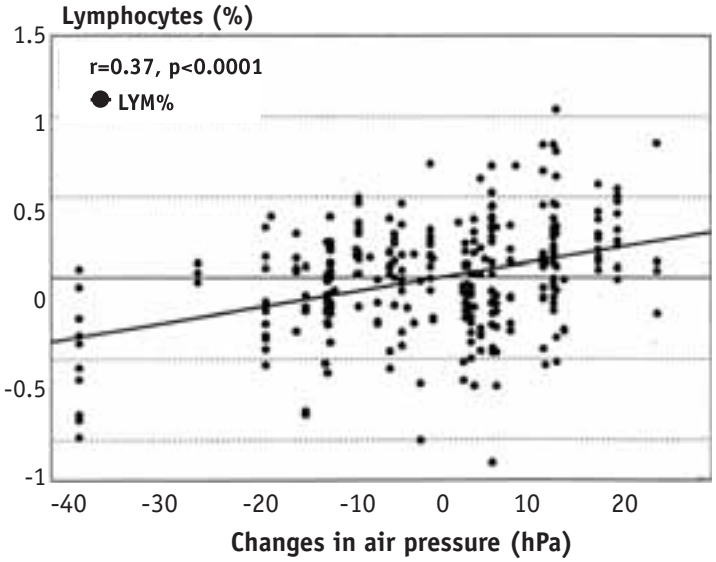

Fig. 4. Regression of percentage of lymphocytes in the peripheral blood on Δ air pressure (changes during the last three weeks).

ship ($r=-0.60$, $p<10^{-4}$) between the $CD4^+/CD8^+$ T cell ratio and ambient temperature (shown are the cyclic signals, computed as described above, in the time series of each of the variables).

By means of bivariate cosinor analyses, we detected significant common rhythms in the time series of ambient temperature and MCV (365 days: $F=218$, $p<0.01$) and MPV (365 days: $F=150$, $p<0.01$), and (after rotation of the time series of ambient temperature by 180°) also with number of RBC (365 days: $F=76.2$, $p<0.01$), MCHC (120 days: $F=36$, $p<0.01$; 90 days: $F=5.7$, $p<0.01$; 60 days: $F=27.4$, $p<0.01$); and number of platelets (365 days: $F=90.1$, $p<0.01$). Figure 6 shows the positive time relationship ($r=0.50$, $p<10^{-4}$) between ambient temperature and MCV (shown are the cyclic signals, computed as described above, in the time series of both variables).

Discussion

The major findings of this study are i) the highly significant linear correlations between immune/hematologic variables in normal human and contemporaneous as well as past (memory effect) weather variables, such as ambient temperature, relative humidity, sunlight duration, wind speed, rainfall duration and air pressure; and ii) significant synchronized seasonal rhythms between RBC and platelet-derived parameters, on the one hand, and ambient temperature, on the other. The results will now be discussed.

On the basis of the regression models used in the present study, it is somewhat difficult to separate the effects of the various present and lagged weather variables on the immune/hematologic data. This problem is generated by the following. i) Interpretation of the relative contribution of the various climatic variables to the variance in the immune/hematologic data is hampered by the presence of multicollinearity in the climatic matrices. ii) The weather data, which are included in the final regression equation, obtained by means of forward automatic regression analyses, show multiple and significant correlations with those that are not included. Therefore, the most conservative interpretation at this stage is that the immune and hematologic variables measured in this study are correlated to short-term fluctuations in atmospheric activity. Nevertheless, it appeared from the automatic step-up regression analyses that some climatic variables were better predictors than others. For example, number (and percentage) of peripheral blood neutrophils and lymphocytes were significantly related to changes in air pressure over the last (three) weeks. Ambient temperature had significant effects on the number or percentage of peripheral blood $CD4^+$ T, $CD8^+$ T, $CD4^+/CD8^+$ ratio, $CD25^+$ T and $CD20^+$ B lymphocytes. Ambient temperature appeared also as one of the most powerful predictors of RBC- and platelet-derived variables, such as number of RBC, Hb, MCV, MCH, MCHC, number of platelets and fibrinogen.

Another major finding of this study is that significant common seasonal rhythms are

Table 2. Results of multiple regression analyses of hematologic variables on present and lagged climatic variables

Hematologic variables	Significant explanatory variables	Multiple regression analyses			
		R^2(%)	F-statistic	df	p-value
RBC	-T2§, +P	5.1	7.8	2/289	0.0008
Hb	_T$ t, +T1	6.4	9.9	2/289	0.0002
Ht	+W4$^{\$}$, +P§, +R3§, +H3	9.9	7.9	4/287	$<10^{-4}$
MCV	+T3* +W4*, +H3$^{\$}$, +W1$^{\$}$, +R3	22.9	17.0	5/286	$<10^{-4}$
MCH	-T*, -W4*, +T3*, -R3*, -S4*, -P*, +T4, +S	39.1	22.7	8/283	$<10^{-4}$
MCHC	-W4*, -T*, -R3*, -P*, -H3*, -S4*, -R1*, +S*, -S1*, -R2*	57.4	24.8	15/276	$<10^{-4}$
RDW	+H2*, +S2*, +T3$^{\$}$, +S§, +R1	19.7	14.0	5/286	$<10^{-4}$
Thrombocytes	+P*, -T4$^{\$}$, +P1§, +P2§	18.4	16.2	4/287	$<10^{-4}$
MPV	-P1*, +S1*, +T3*, -R4$^{\$}$, +W4§, -P§, -P3§, +R	37.8	21.2	8/283	$<10^{-4}$
Fibrinogen	+T*, +H4*, -R3$^{\$}$, +W$^{\$}$, -S3$^{\$}$, -R2$^{\$}$, +R4	24.8	13.2	7/282	$<10^{-4}$

Listed are the results of 10 pooled multiple regression analyses (i.e. pooled over the 26 normal controls) with the hematologic variables as dependent variables and climatic data as explanatory variables. Present and lagged (1, 2, 3 and 4 weeks) values of air pressure (P), sunlight duration (S), wind speed (W), ambient temperature (T), rainfall (R), and relative humidity (H) are entered in pooled multiple regression analyses; 1, 2, 3 and 4 denote a time lag of 1, 2, 3 and 4 weeks, respectively. *p<0.0001; $^{\$}$ p <0.001; § p <0.01; without index: p <0.05.

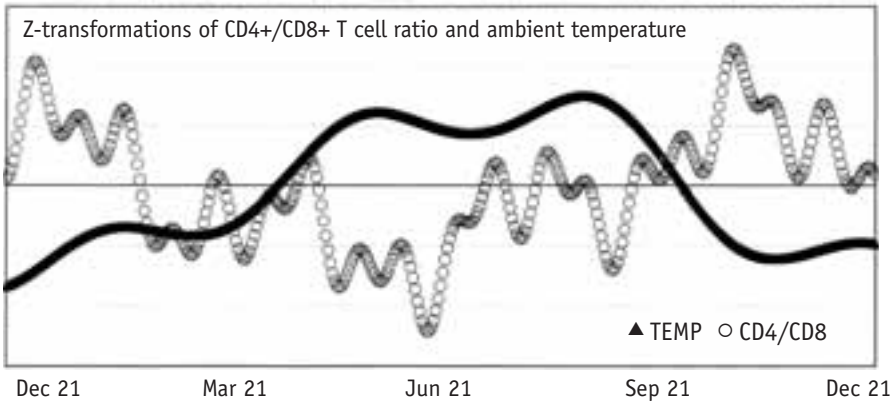

Fig. 5. This figure shows the time relationship between the $CD4^+/CD8^+$ T cell ratio in 26 normal volunteers and ambient temperature (both variables are in z-transformation). There is a significant negative relationship between the cyclic signals in both variables ($r=-0.60$, $p<10^{-4}$).

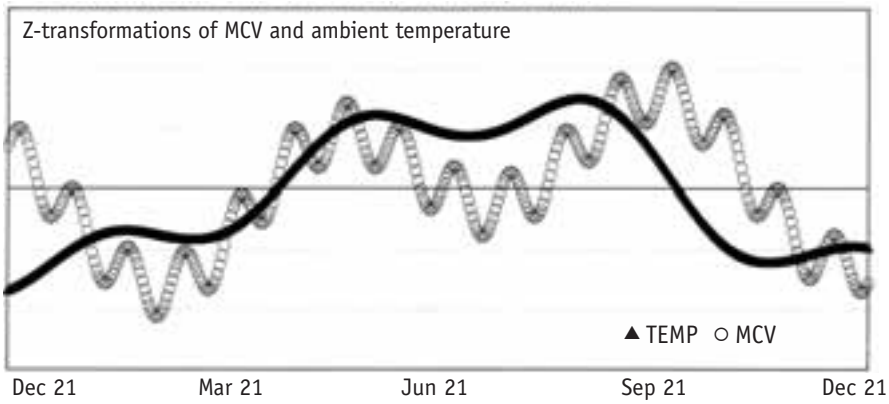

Fig 6. This figure shows the time relationship between mean corpuscular volume (MCV) in 26 normal volunteers and ambient temperature (both variables are in z-transformation). There is a significant positive relationship between the cyclic signals in both variables ($r=0.50$, $p<10^{-4}$).

expressed in the time series of ambient temperature and the $CD4^+/CD8^+$ ratio and number of $CD20^+$ B cells (all inversely related) and $CD25^+$ T cells (positively). Moreover, there were highly significant common seasonal rhythms in ambient temperature and the time series of the numbers of RBC and platelets, MCHC (all inversely related), MCV and MPV (positively related). Our results show that lower ambient temperature may be related to increased numbers of RBC and platelets and Hb concentrations in the peripheral blood and to lower MCV and MPV values. These results are in agreement with a previous report that lower temperature may be accompanied by increases in the counts of peripheral blood platelets and RBC [16]. However, since there are strong correlations between ambient temperature, relative humidity and light-dark span, the most conservative interpretation at this stage is that the seasonal rhythms in immune and, in particular, hematologic variables are strongly related to the seasonal variation in sun insolation parameters, e.g. ambient temperature, light-dark span and relative humidity. Future research in experimental animals should examine which of these variables, alone or in combination, may affect immune and hematologic parameters. There are some spare reports that, in the rodent, exposure to lowered ambient temperature may cause changes in subpopulations of immune cells, isolated from bone marrow or the spleen, and expression of different T (e.g. $CD4^+$) and B (e.g. sIg) cell surface markers [15].

In summary, the above results suggest that short-term changes in the weather not only modulate or affect immune and hematologic measurements in normal human, but also that the seasonal rhythms in these peripheral blood variables may be synchronized by the seasonal rhythms in sun insolation variables. These findings lend support to the hypothesis that the seasonal time structure in immune and hematologic variables in human may, in part, be entrained or synchronized by climatic factors. On the other hand, the results of the present study do not rule out the possibility that the seasonal time structure in both immune and hematopoietic systems in human are also genetically determined. This hypothesis is corroborated by findings that in experimental animals, circannual rhythms in T cell immunity occur even when climatic data are held constant and that there is a genetic difference in the circannual production of antibodies [11, 26].

Although our results clearly show that there are significant time relationships between the weather and immune/hematologic variables, they do not provide further information on the exact nature of these relationships. Indeed, since we measured the immune/hematologic variables 08:00 a.m. morning blood samples, we cannot rule out that the relationships observed here are due to a putative climate-induced modulation of possible circadian rhythms in the dependent variables (e.g. shift in mesor or acrophase). Indeed, previous findings suggest a seasonal modulation of circadian rhythms in number of lymphocytes, $CD4^+$ T, and $CD4^+/CD8^+$ cell ratio, but not in number of RBC, Hb or Ht [5, 8]. Thus, the significant time relationships between climate and immune- (and maybe platelet-) related data may be due to effects of the weather on circadian rhythm parameters (e.g. shift in acrophase or mesor), and/or to effects on the main daily values of these variables. The time relationships between climatic and RBC-related variables are probably due to effects of the weather on the mean 24 hr values of those variables.

It is tempting to hypothesize that the time relationships between the yearly variations in the weather and in immune/hematologic data are related to the yearly variations in immune-related and hemostasis-related disorders. There is strong evidence of a yearly variation in the incidence of infectious disorders with peaks occurring in spring or in late winter [2, 5, 27–31]. There is also a true seasonality in the symptomatology, incidence and mortality due to certain types of neoplasia, such as breast cancer, with peaks in spring-summer [5, 32–34]. Thus, changes in the number of peripheral blood T and B lymphocytes—related to the seasonal variation in weather variables—could alter the susceptibility/resistance to some immune disorders. It is now well established that hemorrhagic stroke, peripheral embolism, and coronary and cerebral thrombosis show a peak incidence in winter [35–39]. Other findings suggest that short term fluctuations in ambient temperature are related to incidence of these disorders [38, 40]. A statistically negative correlation between ambient temperature and mortality from cerebro-cardiovascular disease [40] and incidence of intracerebral hemorrhage and cerebral infarction [41] has been described. Gill et al. [38] reported a strong association between the chilling effect of the atmosphere and incidence of subarachnoid hemorrhage and thromboembolic brain infarction. Hence, it may be hypothesized that climate-induced alterations (e.g. temperature) in biological rhythms, which modulate hemostasis (e.g. number of platelets, fibrinogen), may be related to the seasonal changes in the incidence of hemostasis-related disorders. In addition, from our results, it may appear that the increased number of RBC in colder periods of the year may play a role, since this phenomenon may promote platelet adhesion [16]. Consequently, it may be hypothesized that cli-

mate-induced changes in susceptibility/resistance rhythms in immune or hematologic functions across the seasons could contribute to an increased incidence of infectious disorders, some cancers or neoplasias or of hemostasis-related disorders in some periods of the year.

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