Clinical Immunology
Vol. 98, No. 1, January, pp. 104–108, 2001
doi:10.1006/clim.2000.4952, available online at http://www.idealibrary.com on IDEAL®

Inverse Relationship between Neopterin and Immunoglobulin E

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Polarized human T helper (Th) cells play a key role in the network of the specific immune system compartments. Cell-mediated immune response depends on activation of Th1-type cells, typically producing and releasing interferon-γ and interleukin-2, whereas activation of Th2-type cells and production of cytokines such as interleukin-4, -5, and -10 are involved in humoral immune response and the production of immunoglobulins. Increased amounts of neopterin are produced during the Th1-type immune response by human monocytes/macrophages upon stimulation with the Th1-derived cytokine interferon-γ, and thus the determination of neopterin concentrations allows us to monitor Th1-type immune response. We compared serum concentrations of neopterin with immunoglobulin E (IgE), a typical product of the Th2-type immune response, in order to examine the relationship between Th1-type and Th2-type immune system stimulation in 709 healthy outpatients, who visited the physician's office for a medical health checkup. Eleven percent presented with serum neopterin concentrations >8.7 nmol/L; 26% had increased serum concentrations of IgE (>100 kIU/L). There existed an inverse correlation between serum neopterin and IgE concentrations (Spearman's rank correlation coefficient: $r_s = -0.100$; $P < 0.01$) which was stronger when excluding data ≤8.7 nmol/L neopterin and ≤100 kIU/L IgE ($n = 246$; $r_s = -0.519$; $P < 0.0001$). Data indicate that increased serum neopterin concentrations are associated with low serum IgE and increased serum IgE with low serum neopterin concentrations. This finding fully agrees with the current understanding that in humans the activation of Th1 and Th2 cell-mediated immune responses are down-regulating each other.

Key Words: **neopterin; immunoglobulin E; T helper cells.**

INTRODUCTION

Polarized human T helper (Th) cells, namely, Th1 and Th2 cells, play a key role in the network of the specific immune system (1). It is now assumed that antigen presenting cells, like dendritic cells, which inform naive Th cells about invading pathogens, prime naive Th cells by the amount of interleukin (IL)-12 they produce to differentiate to Th1 or Th2 cells (2, 3). Th1 cells produce interferon-γ (IFN-γ) and IL-2, thereby promoting cell cytotoxicity mediated by cytotoxic T cells (4). Upon stimulation by IFN-γ, preferentially human monocytes/macrophages produce and release large amounts of neopterin (5), 6-D-erythro-1′,2′,3′-trihydroxypropyl-pterin, which is synthesized from guanosine triphosphate (GTP) by GTP-cyclohydrolase I (EC 3.5.4.16) (6). In humans, increased concentrations of neopterin in serum and urine have been found in viral infections, including human immunodeficiency virus type 1 (HIV-1), various malignant disorders, and autoimmune diseases, and during allograft rejection episodes (7–13). Significant associations between enhanced blood concentrations of neopterin and IFN-γ have also been obtained in patients (14), and determination of neopterin concentrations has turned out to be a sensitive and useful way for monitoring the activation of Th1-type immune response (8). On the other hand, Th2 cells are characterized to mainly produce interleukins-4, -5, -6, -9, -10, and -13, thereby supporting humoral immune response (4) which is associated with hyperproduction of immunoglobulins, e.g., immunoglobulin E (IgE). A cross-regulatory influence exists between Th1 and Th2 cell subpopulations, down-regulating each other when activated. This is evident from *in vitro* experiments, in which cytokines IL-2 and IFN-γ were demonstrated to inhibit development and activation of Th2 cells and interleukin-4 and -10 inhibited Th1 cells. Also, neopterin production by human peripheral blood mononuclear cells upon stimulation with the Th1-type cytokine IFN-γ is suppressed by Th2-type cytokines IL-4 or IL-10 (15), supporting the concept that Th1- and Th2-type immune responses will not occur in parallel but more probably alternatively.

Most of the available data on the cross-regulatory influence between the two Th cell subpopulations so far comes from *in vitro* studies. The present study was designed to examine the possible relation of Th1 and Th2 cell response in humans by comparing serum concentrations of neopterin, a typical product of Th1-type

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immune response, with concentrations of IgE, a typical product of Th2-type immune response.

MATERIALS AND METHODS

Study population. There were 709 healthy outpatients, 274 males and 435 females, who visited the physician's office for a medical health checkup but not feeling sick and were studied. The patients' ages varied from 11 to 93 years (median age, 41 years, Table 1).

Blood collections and measurements. Blood samples were drawn after an overnight fast. The blood was allowed to clot at room temperature, and serum was obtained by centrifugation at 1500*g* for 15 min. All analyses were performed within 1 day after blood collection. Serum neopterin was measured by a commercially available radioimmunoassy (BRAHMS Diagnostica, Berlin, Germany) with a sensitivity of 1 nmol/L neopterin and an interassay coefficient of variation ranging from 4.7 to 8.5%. Upper limits of the normal (95th percentiles), depending on age, ranged from 8.7 nmol/L (19–75 years) to 13.5 nmol/L (below 18 years) and 19.0 nmol/L (above 75 years) as described earlier (13).

Serum IgE was measured by an immunometric assay with an IMMUNITE 2000 Analyzer and a commercial kit (Immulite 2000 Total IgE, Diagnostic Products Corp., Los Angeles, CA), with a sensitivity of 1 kIU/L IgE and an interassay coefficient of variation ranging from 5.1 to 6.7%, the laboratory's normal range for adults being below 100 kIU/L.

Statistical analysis. Correlation between variables were assessed by the nonparametric Spearman's rank correlation technique, since the distributions of the observed values were generally non-Gaussian. This was done for all data and for data excluding those with serum neopterin ≤8.7 nmol/L (=95th percentile of healthy adults with ages ranging from 19 to 75 years) and IgE ≤ 100 kIU/L. For the latter also a least square linear regression line of the logarithmically transformed data was calculated, resulting in a geometric curve after retransformation. Regression analysis was done by the program BMDP1R (BMDP Statistical Software, 1990 edition, University of California Press). Frequencies within groups were compared by Fisher's exact test.

RESULTS

Ten percent of the patients studied presented with elevated serum neopterin concentrations; 26% had elevated serum IgE concentrations (Table 1). From approximately half of the subjects, standard prick skin test results were available, and of 79 atopics (mostly perennial symptoms), 47 (59.5%) had IgE > 100 kIU/L compared with 32 (40.5%) with IgE ≤ 100 kIU/L ($P < 0.001$; Fisher's exact test). The incidence of increased neopterin concentrations, however, did not differ between atopics and non-atopics.

As seen from Fig. 1, increased serum neopterin concentrations were preferentially associated with low serum IgE and increased serum IgE with low serum neopterin concentrations. There were only 18 subjects presenting with slightly increased neopterin and IgE concentrations at the same time. Associations between the investigated variables assessed by Spearman's rank correlation coefficients are shown in Table 2. There was an inverse correlation ($n = 709$; $r_s = -0.100$; P 0.0078) between serum IgE and serum neopterin concentrations. On excluding data with neopterin ≤8.7 nmol/L and IgE ≤ 100 kIU/L, a strong inverse correlation ($n = 246$; $r_s = -0.519$; $P < 0.0001$) can be seen. For the latter data also a least square regression line of the logarithmically transfomed data was calculated, resulting in the following geometric curve after retransformation: IgE × Neopterin$^{1.92}$ = 4452 (see Fig. 1). Thereby, about 37% of the data points could be estimated, as calculated from the squared regression coefficient ($n = 246$; $R = 0.605$; $P < 0.0001$).

DISCUSSION

Data show an inverse relationship between serum neopterin and serum IgE concentrations, supporting the concept that activated Th1- and Th2-type immune responses are cross-regulated and thus will not occur in parallel but more probably excluding each other.

TABLE 1

Baseline Characteristics of the Study Subjects ($n = 709$: 274 Males and 435 Females)

Characteristics	First quartile	Median value	Third quartile	Range	Above reference range: *n* (%)
Age, years	33.1	40.6	52.6	10.7–92.9	
Immunoglobulin E, kIU/L	11.7	34.0	103.7	≤1–2136	184 (26.0)
Neopterin, nmol/L	4.5	5.4	6.7	≤1–36.8	71 (10.0)

Note. Reference ranges: neopterin ≤13.5 (<19 years), ≤8.7 (19–75 years), ≤19.0 (>75 years) nmol/L; immunoglobulin E ≤100 kIU/L.

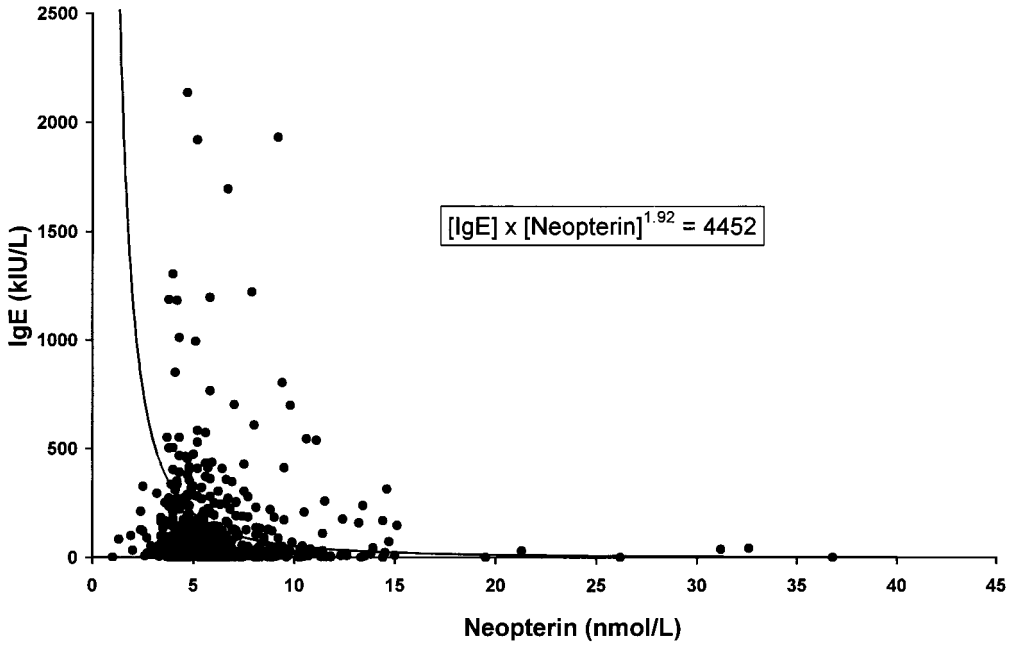

FIG. 1. Scatter plot of serum neopterin versus serum IgE concentrations of the investigated patients. The geometric curve IgE · Neopterin$^{1.92}$ = 4452 (n = 246; R = 0.605; $P < 0.0001$) was calculated after excluding values with neopterin ≤8.7 nmol/L and IgE ≤100 kIU/L.

This inverse correlation was stronger when restricting the study population to those with neopterin or IgE outside the reference range. This supports the assumption that only in situations of immune stimulation did the concept of alternatively activated Th cell subpopulations become detectable.

Increased concentrations of neopterin in humans are produced by the immune system in response to viral infections, including human immunodeficiency virus type 1 (HIV-1), in response to various malignant disorders, during autoimmune diseases, and during allograft rejection, and they are indicative for cell-mediated (Th1-type) immune response (7–13). The increased production of neopterin is associated with an enhanced formation of free radicals by the immunocompetent cells (16) and there are data accumulating that neopterin derivatives themselves are capable of modulating the oxidative capacity of several reactive metabolites, such as hydrogen peroxide, hypochlorous acid, or peroxynitrite (17, 18). With this background, neopterin concentrations can also be regarded as an indirect measure of oxidative stress elicited during immune reaction (19). This assumption is further substantiated by *in vivo* data: e.g., in demented patients an inverse correlation has been found between intrathecal concentrations of α-tocopherol and neopterin (20).

Recent data already have shed some light on the cross-regulatory impact of Th1- and Th2-type immune response on the production of neopterin. *In vitro*, neopterin production by human peripheral blood mononuclear cells upon stimulation with the Th1 cytokine IFN-γ could be suppressed by typical Th2 cytokines like IL-4 or IL-10 (15). And also histamine—a mediator of allergic disease mainly released from stimulated mast cells—was found to down-regulate neopterin production in peripheral blood mononuclear cells (21).

TABLE 2

Spearman's Rank Correlations of Investigated Characteristics

		Spearman's rank correlation coefficient			
	n	Value	95% confidence interval		P value
Neopterin versus IgE					
All subjects	709	−0.100	−0.174	−0.024	0.0078
Subjects with normal tests (Neopterin >8.7 nmol/L or IgE >100 kIU/L)	246	−0.519	−0.608	−0.419	<0.0001

The data of this study were generated from a healthy population, in which no clear-cut explanation for the increased neopterin or IgE concentrations was apparent. Most likely, clinically silent infectious diseases may have played a role. In general, acute viral infections are accompanied by high neopterin levels reflecting activated cell-mediated immune response and involvement of Th1-type cells (8). On the contrary, in acute bacterial infections preferentially low neopterin concentrations are observed which are indicative of the absence of a Th1-type immune response, in agreement with a Th2-type immune response when antibody production is activated. Only in chronic bacterial infections, however, may concentrations of neopterin rise (22).

In the course of HIV-1 infection the role of Th1 and Th2 subsets is somewhat conflicting. On the one hand, some studies show an IgE overproduction (23, 24), which would be in line with the concept of a shift occurring from Th1-type toward Th2-type immune response during the course of HIV-1 infection (25). On the other hand, a continuing increase of neopterin and IFN-γ concentrations in the blood was demonstrated in patients with HIV-1 infection (7, 9, 26). The latter finding is in sharp contrast with the view of a shift away from Th1-type toward Th2-type immune response; rather, it suggests an increase in the Th1-type cytokine IFN-γ. On the mRNA level, increased expression of IFN-γ and IL-10 was observed in $CD4^+$ T cells, whereas the expression of IL-2 and also of IL-4 was hardly detectable in HIV-1 infection (27–29). In summary, it seems that there occurs an activation of both Th compartments alternatively or a shift to the Th0 phenotype takes place (4). Other *in vivo* studies seem to confirm this view. Also, in acute episodes of graft-versus-host disease after human allogeneic bone marrow transplantation a significant positive correlation between plasma neopterin levels and the Th2-derived cytokine IL-10 was found (30).

Our study of healthy individuals indicates that increased serum neopterin concentrations are associated with low serum IgE, and increased serum IgE with low serum neopterin concentrations, which agrees with the current understanding that in humans Th1 and Th2 cell-mediated immune responses are down-regulating each other. Nevertheless, further studies comparing, e.g., neopterin and IgE production in parallel in selected populations of patients, such as HIV-1-infected individuals, will be necessary to elaborate the possible broader relevance of this phenomenon in more detail.

ACKNOWLEDGMENTS

This work was supported by the Austrian Funds "Zur Förderung der wissenschaftlichen Forschung," Project 14154Med, and the Austrian Federal Ministry of Social Affairs and Generations.

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Received May 31, 2000; accepted with revision September 5, 2000; published online November 28, 2000