In Vitro Production of Antibodies to Histones in Patients Receiving Chronic Procainamide Therapy

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ABSTRACT. ***Objective.*** Procainamide related autoimmunity is characterized by the production of antibodies to histones and, in particular, to the H2A-2B dimer. We evaluated *in vitro* production of antibodies to total histones and the H2A-2B dimer by peripheral blood mononuclear cells (PBMC) from patients chronically exposed to procainamide and related this to *in vivo* production, and assessed possible immunostimulatory response by the postulated reactive metabolite procainamide hydroxylamine (PAHA) using PAHA conjugated autologous erythrocytes.

Methods. We evaluated *in vitro* spontaneous and mitogen induced production of histone antibodies by PBMC from 26 asymptomatic patients, who were chronically receiving procainamide, in the presence and absence of PAHA conjugated autologous erythrocytes. Correlations with *in vivo* production were sought.

Results. PBMC from 9 patients revealed significant spontaneous production of histone antibodies, of whom 2 developed procainamide related lupus within 2 mo of the evaluation. There was a significant increase in *in vitro* production of antibodies to total histones by PBMC that had been cultured in the presence of PAHA-autologous erythrocyte conjugates, but in the absence of mitogens, from 15 (65%) of 23 patients, and of antibodies to H2A-2B by cells from 10 (42%) of 24 patients. Patients' cells that were co-cultured with PAHA-erythrocyte conjugates produced significantly greater amounts of antibodies to both total histones ($p = 0.03$) and the H2A-2B dimer ($p = 0.009$) compared with those cultured alone. Co-culture with similarly pretreated erythrocytes also resulted in a significant increase in the production of antibodies to total histones ($p < 0.001$), but not to the H2A-H2B dimer, by cells from controls.

Conclusion. Some patients receiving chronic procainamide therapy have spontaneous production of histone antibodies. Co-culture with PAHA-erythrocyte conjugates resulted in significantly greater production, suggesting an immunomodulating effect by this metabolite. (J Rheumatol 2001; 28:1992–8)

Key Indexing Terms:
HISTONE ANTIBODIES DRUG RELATED LUPUS PROCAINAMIDE

Drug related autoimmunity is characterized by the presence of antinuclear antibodies (ANA) with reactivity primarily to the nucleosome and in particular to histones. Procainamide is the drug most commonly implicated in the development of drug related autoimmunity although the pathogenetic mechanism remains to be defined. Procainamide related autoimmunity is characterized by the development of histone antibodies directed to the H2A-2B dimer complex[1–3].

Results of studies evaluating the effects of procainamide and its metabolites on the immune response have been inconsistent. Studies that have evaluated the *in vitro* effects of procainamide on lymphocytes from healthy individuals have yielded discrepant results, with some reporting an increase in the generation of immunoglobulin (Ig) secreting cells[4,5] and others reporting no significant effect on IgG secretion[6,7].

Studies evaluating *in vitro* pokeweed mitogen stimulated Ig secretion by lymphocytes from patients receiving procainamide have also yielded conflicting results, one reporting B cell hyporesponsiveness and the other an increase in IgG secretion[8,9]. However, *in vitro* IgG secretion by unstimulated cells from patients receiving procainamide did not differ significantly from patients with systemic lupus erythematosus (SLE) or controls[9]. In contrast, Forrester, *et al*[10] reported a significant increase in spontaneous IgG production by B lymphocytes obtained from patients with procainamide related lupus compared with those from control groups, which included a group of 6 asymptomatic patients receiving procainamide and a group of young healthy controls. An increase in spontaneous IgM production by B lymphocytes from both symptomatic and asymptomatic patients receiving procainamide was also noted, but it did not reach statistical significance. These data suggest that spontaneous production of IgG antibodies may be associated with the development of drug related lupus. The reactivity of the IgG antibodies was not evaluated.

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Spontaneous production of antibodies to histones by peripheral blood mononuclear cells (PBMC) from a large subset of patients with SLE was correlated with both disease activity and levels of antihistone antibodies in patients' sera, suggesting that it reflected *in vivo* production[11]. It was independent of polyclonal B cell activation, measured by total *in vitro* Ig synthesis in culture, suggesting selectivity of the autoantibody response in these patients. Procainamide related autoimmunity is believed to be triggered by chronic exposure to the medication, or more likely a metabolite. One possible pathogenic mechanism is the generation of an immune response by one of the metabolites, which would likely be characterized by selectivity of its associated autoantibody response. Rubin and colleagues have reported that injection of the hydroxylamine metabolite of procainamide into the thymus of healthy mice induced the formation of autoantibodies to histones (H2A-2B)[12].

Evidence suggests that procainamide related autoimmunity may be caused by a N-oxidized reactive metabolite of procainamide, procainamide hydroxylamine (PAHA), which rapidly binds to hemoglobin after it is oxidized to the nitroso-metabolite by peroxidases in erythrocytes[13-16]. This interaction has been postulated to be important in mediating the immunomodulating effect of PAHA. Although PAHA has not been identified in the circulation, nitroprocainamide, formed as an end product of oxidation of PAHA *in vitro*, has been detected in the urine of patients receiving chronic procainamide therapy, suggesting that PAHA is formed *in vivo* in patients receiving this medication[17,18]. Adams, *et al* reported that pre-incubation of whole blood from healthy individuals with low concentrations of the metabolite PAHA augmented the response to mitogen and Ig secretion, but that pretreatment with high concentrations resulted in immunosuppression[7]. However, the suppressive effect of PAHA was reduced if erythrocytes were removed or if hemoglobin was converted to carboxyhemoglobin, suggesting that binding of PAHA to hemoglobin may be a prerequisite. A possible effect on the production of histone antibodies was not evaluated.

We evaluated *in vitro* production of antibodies to total histones and the H2A-2B dimer by PBMC from patients chronically exposed to procainamide and related this to *in vivo* production, and investigated a possible immunostimulatory response induced by the postulated reactive metabolite, PAHA, using PAHA conjugated autologous erythrocytes.

MATERIALS AND METHODS

Patients. Twenty-six patients who were receiving chronic procainamide treatment for cardiac dysrhythmias agreed to participate. These patients were part of a longitudinal study evaluating the development of procainamide related autoimmunity[1]. They consisted of 23 Caucasians and 3 African Americans, all male. The average age was 63.6 years (range 50 to 76 yrs). The average dose of procainamide was 2894 mg (range 750 to 4500 mg) and mean duration of treatment with procainamide was 44.3 months (range 3 mo to 9 yrs).

Controls. Fourteen healthy medication-free Caucasian individuals served as controls, 7 men and 7 women with a mean age of 42 years (range 30 to 63 yrs). Since the assays were performed over a period of weeks, depending on the availability of the patients, blood was obtained from some of these individuals for control purposes on more than one occasion. At least one control was included for each assay. Results were similar for both men and women. Blood was also obtained from these individuals for use in preliminary experiments to determine the optimal conditions for *in vitro* production of histone antibodies.

Serologic assays. Sera were removed from blood samples obtained from patients and controls and stored at –20°C.

Cellular assays. Freshly heparinized blood was placed on a 10% dextran gradient and incubated at 37°C for about 1 h. The leukocyte-rich plasma was aspirated and washed 3 times with Hanks' balanced salt solution (HBSS; Gibco Laboratories, Grand Island, NJ, USA) supplemented with antibiotic/antimycotic mixture (Gibco) and gentamycin (Gibco). PBMC were then isolated by Ficoll-Hypaque density gradient centrifugation (Pharmacia, Piscataway, NJ, USA). Cells retrieved from the interface were washed 3 times with HBSS supplemented with antibiotic/antimycotic mixture and gentamycin and resuspended in final medium consisting of RPMI-1640 solution (Gibco) containing antibiotic/antimycotic mixture, gentamycin, and 10% heat inactivated fetal calf serum (Gibco). Cell viability was determined by use of the Trypan blue exclusion assay. In these experiments, cell viability was > 99%.

Experiments were initially performed to determine the optimal conditions for *in vitro* production of histone antibodies using PBMC from healthy, medication-free individuals. Although it is rare for histone-specific B cells from healthy individuals to produce histone antibodies spontaneously, studies have confirmed the production of these antibodies by PBMC from individuals following mitogen stimulation[11].

In our initial experiments, various concentrations of PBMC ($1–5 \times 10^6$ cells/ml) were cultured in combination with different concentrations and combinations of mitogens over varying time periods (7–14 days). By these means, it was determined that optimal conditions consisted of a 14 day culture of PBMC, suspended at a concentration of 5×10^6 cells/ml, with pokeweed mitogen (Gibco), at a final concentration of 1:100, and *Salmonella minnesota* lipopolysaccharide (LPS; Sigma Chemical Co., St. Louis, MO, USA) at a final concentration of 20 μg/ml. These were the conditions employed for this assay. Cells were therefore incubated at a concentration of 5×10^6/ml in sterile round bottom glass tubes either alone or with mitogens at 37°C in a 5% CO_2 humidified atmosphere for 14 days. Supernatants were then retrieved and placed at –20°C until assayed for antibodies to histones and the H2A-2B dimer complex.

Preparation of PAHA-erythrocyte conjugates. In the cases of patients and controls where there were adequate numbers of PBMC, additional cultures were set up to which 0.05 ml of PAHA-autologous erythrocyte conjugates were added alone or in combination with mitogens to assess for an antigen driven response. PAHA was prepared from *p*-nitroprocainamide by Dr. J. Wheeler, Department of Chemistry, University of Cincinnati, as described[19]. Confirmation of the compound was performed by H nuclear magnetic resonance, infrared, and mass spectra tests. The hydroxylamine metabolite was stored under nitrogen at –80°C until used. After the leukocyte-rich plasma had been removed from the dextran gradient, the remaining erythrocytes were washed three times with sterile saline solution. Procainamide hydroxylamine was then added to give a final concentration of 10 μg/ml. The erythrocyte suspension was diluted with sterile saline to give a total volume of 1 ml and incubated at 37°C for 30 min. The erythrocytes were then washed 3 times with HBSS. After removal of HBSS, 50 μl of erythrocyte concentrate were added to the tubes containing the PBMC to give a final concentration of 5% erythrocytes.

Assay to detect histone antibodies. Patients' sera were assayed for antibodies to histones and the H2A-2B dimer using an ELISA, as described[1], and the results were compared to those of the *in vitro* assays. Plates were coated with histones and with H2A-2B dimer complex at concentrations of

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2.5 μg/ml and 2 μg/ml, respectively. *In vitro* production of these histone antibodies was assessed using a modification of this assay. The supernatants retrieved from the cell cultures were used undiluted and the peroxidase conjugated anti-human IgG was diluted 1:1000 in the antiimmunoglobulin diluent. For each of these assays, samples were run in triplicate.

Statistical analysis. Statistically significant differences in the *in vitro* production of histone antibodies were evaluated by comparing the mean optical density (OD) value and standard deviation (SD) for each patient with those of the control group using Student's t test. Differences in frequencies between groups were analyzed using either chi-square or Fisher's exact 2 tailed 2 × 2 test, as appropriate. Mann-Whitney rank sum method was used for comparisons between groups and correlations were sought using Spearman's rank correlation method. A significant statistical difference was accepted at $p < 0.05$.

RESULTS

Patients. Twenty-six patients receiving longterm procainamide treatment were evaluated. No patient had clinical evidence of lupus at the time of evaluation. However, 4 patients subsequently developed procainamide related lupus. Twenty-four (92%) patients had antinuclear antibodies, 19 (73%) had IgG antibodies to total histones, and 16 (62%) had IgG antibodies to the H2A-2B dimer complex (Table 1).

Spontaneous antibody production. Spontaneous *in vitro* production of antibodies to total histones was significantly increased in the cultures of PBMC from 9 (35%) of the 26 patients compared to controls (Table 2). Seven had antibodies to total histones in their sera, although no statistical correlation with *in vitro* production was found ($p > 0.05$). The mean OD value ± SD of 0.0722 ± 0.08 for the patient group was significantly higher than that of 0.03 ± 0.02 for controls ($p < 0.001$, Mann-Whitney test) (Figure 1). Data points for patients and controls are shown in Figure 2.

There was a significant increase in the *in vitro* production of antibodies to the H2A-2B dimer complex by PBMC from 3 (12%) of the 26 patients, of whom one also had antibodies to the H2A-2B dimer in his serum (Table 2). The mean OD value ± SD of 0.046 ± 0.04 for the patient group did not differ significantly from that of 0.036 ± 0.02 for the controls ($p = 0.523$ by Mann-Whitney). The data points for patients and controls are shown in Figure 3.

Mitogen stimulated production. There was a significant increase in the *in vitro* mitogen stimulated production of antibodies to total histones by PBMC from 14 (54%) of the 26 patients (Table 2). Twelve of these patients had antibodies to total histones in their sera, although there was no significant association with *in vitro* production of histone antibodies by the respective patients' cells ($p = 0.12$ for both types of antibodies). The mean OD value ± SD of 0.136 ± 0.133 for the patient group was significantly increased compared with that of 0.079 ± 0.1 for the controls ($p = 0.004$ by Mann-Whitney). These mean values were significantly greater than the respective values for those cells cultured in the absence of mitogens ($p < 0.001$ for the patients and

Table 1. Patient characteristics.

No. of patients	26
Ethnicity	23 Caucasian, 3 African-American
Mean age	63.6 yrs (range 50 to 76)
Average procainamide dose	2894 mg (range 750 to 4500)
Mean duration of procainamide treatment	44.3 mo (range 3 mo to 9 yrs)
No. of patients with ANA	24 (92%)
No. of patients with serum antibodies to total histones	19 (73%)
No. of patients with serum antibodies to H2A-2B dimer	16 (62%)

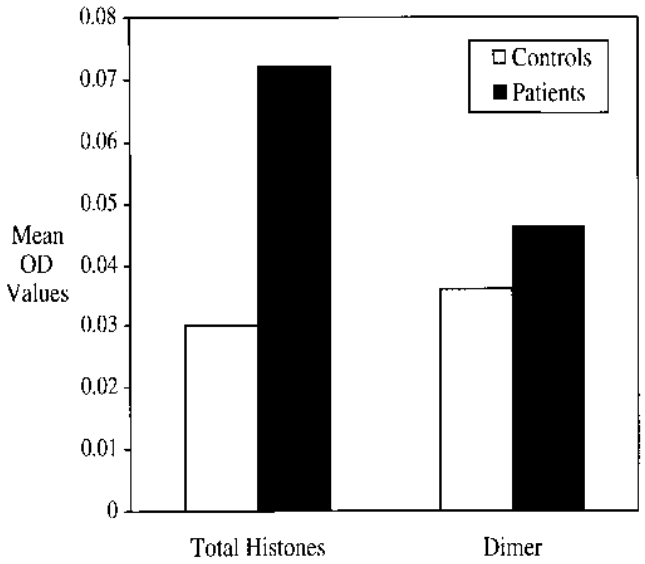

Figure 1. Spontaneous *in vitro* production of antibodies to total histones and to the H2A-2B dimer by PBMC from patients receiving chronic procainamide and controls. Culture conditions and antibody assays as described in Materials and Methods. There was a significant increase in the production of antibodies to total histones by PBMC of patients compared with controls ($p < 0.001$, Mann-Whitney).

Table 2. *In vitro* production of histone antibodies by PBMC from patients receiving chronic procainamide therapy. PBMC from patients were cultured alone (spontaneous), with mitogens, with PAHA-autologous erythrocyte conjugates (PAHA-RBC), or with PAHA-RBC and mitogens. *In vitro* production of antibodies to total histones and to the H2A-2B dimer was determined by modified ELISA.

Culture Conditions	No. of Patients with Significant* *in vitro* Antibody Production to Total Histones (%)	Dimer (%)
Spontaneous	9/26 (35)	3/26 (12)
Mitogens	14/26 (54)	5/26 (19)
PAHA-RBC	15/23 (65)	10/24 (42)
PAHA-RBC + mitogens	10/24 (42)	10/22 (45)

* Significant production was determined by comparing the mean OD ± SD of individual patients with those of the control group using Student's t test. A p value of < 0.05 was considered to be significant.

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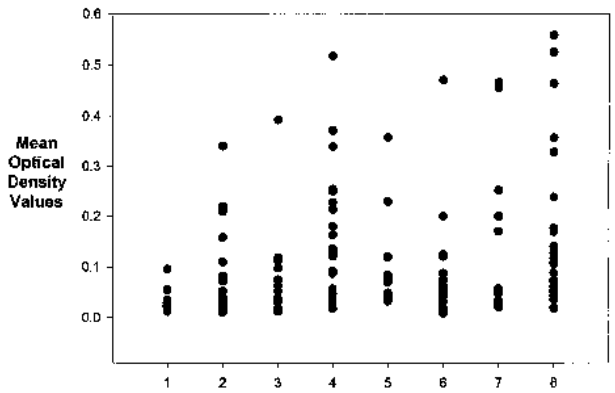

Figure 2. In vitro production of antibodies to histones by PBMC obtained from patients receiving procainamide and controls that were cultured with and without mitogens in the presence and absence of PAHA conjugated autologous erythrocytes (PAHA-RBC). Supernatants were analyzed for antibodies to total histones by ELISA. Each point represents data from a different patient or control. Lanes 1, 3, 5, 7 represent values obtained from supernatants of PBMC from controls cultured alone (1), with mitogens (3), with PAHA-RBC (5), and with mitogens and PAHA-RBC (7). Lanes 2, 4, 6, 8 represent values obtained from supernatants of PBMC from patients cultured alone (2), with mitogens (4), with PAHA-RBC (6), and with mitogens and PAHA-RBC (8).

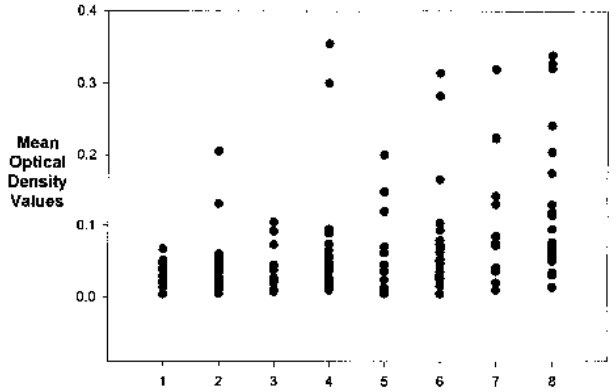

Figure 3. In vitro production of antibodies to histones by PBMC from patients receiving procainamide and controls that were cultured with and without mitogens in the presence and absence of PAHA conjugated autologous erythrocytes (PAHA-RBC). Supernatants were analyzed for antibodies to the H2A-2B dimer by ELISA. Each point represents data from a different patient or control. Lanes 1, 3, 5, 7 represent values obtained from supernatants of PBMC from controls cultured alone (1), with mitogens (3), with PAHA-RBC (5), and with mitogens and PAHA-RBC (7). Lanes 2, 4, 6, 8 represent values obtained from supernatants of PBMC from patients cultured alone (2), with mitogens (4), with PAHA-RBC (6), and with mitogens and PAHA-RBC (8).

$p = 0.005$ for controls by Mann-Whitney), presumably as a result of a mitogenic stimulatory effect.

There was a significant increase in *in vitro* mitogen stimulated production of antibodies to the H2A-2B dimer by the cells from 5 (19%) patients, of whom 3 also had these antibodies in their sera. There was no significant difference between the mean OD values for the patients and controls ($p = 0.417$ by Mann-Whitney).

Effect of co-culture with PAHA-autologous erythrocyte conjugates. Where there were sufficient amounts of PBMC, additional cultures were set up to which PAHA-autologous erythrocyte conjugates were added, alone or in combination with mitogens, to evaluate for an immunostimulatory effect by PAHA.

There was a significant increase in the *in vitro* production of antibodies to total histones by PBMC that had been cultured in the presence of PAHA-autologous erythrocyte conjugates, but in the absence of mitogens, from 15 (65%) of 23 patients compared with controls (Table 2). Fourteen of these 15 patients also had histone antibodies in their sera, although no significant association with *in vitro* production was found.

Significant levels of antibodies to H2A-2B were produced by cells from 10 (42%) of 24 patients that were co-cultured with PAHA-autologous erythrocyte conjugates. Sera from 5 of these 10 patients also contained antibodies to the H2A-2B dimer, although no significant association with *in vitro* production was found.

To evaluate for an immunostimulatory effect by the PAHA metabolite, the *in vitro* production of antibodies by cells cultured in the presence of PAHA conjugated autologous erythrocytes, but without mitogens, was compared to spontaneous *in vitro* production. Patients' cells that were co-cultured with PAHA-erythrocyte conjugates produced significantly greater amounts of antibodies to both total histones ($p = 0.03$) and the H2A-2B dimer ($p = 0.009$) compared with those cultured alone (Figure 4). Co-culture with similarly pretreated erythrocytes also resulted in a significant increase in the production of antibodies to total histones ($p < 0.001$), but not to the H2A-2B dimer, by cells from the controls (Figure 5).

Following mitogen stimulation, cells from 10 (42%) of 24 patients that were cultured with PAHA-erythrocyte conjugates showed significant production of antibodies to total histones (Table 2). Similarly, 10 (45%) of 22 patients' cell cultures showed significant production of antibodies to H2A-2B. Seven of 10 patients each had the respective antibodies in their sera, but no statistically significant correlation between *in vivo* and *in vitro* production of either of these antibodies was found ($p > 0.05$).

The addition of PAHA conjugated erythrocytes to mitogen stimulated cultures resulted in statistically significant increases in the mean *in vitro* production of antibodies to the H2A-2B dimer (Figure 4), but not to total histones, by patients' cells ($p < 0.001$, Mann-Whitney) and of antibodies to both total histones ($p < 0.001$, Mann-Whitney) (Figure 5) and the H2A-2B dimer ($p = 0.022$, Mann-Whitney) by controls' cells.

Thus, the addition of PAHA conjugated autologous erythrocytes to both unstimulated and mitogen stimulated cultures of PBMC from patients receiving procainamide was associated with an increase in the production of anti-

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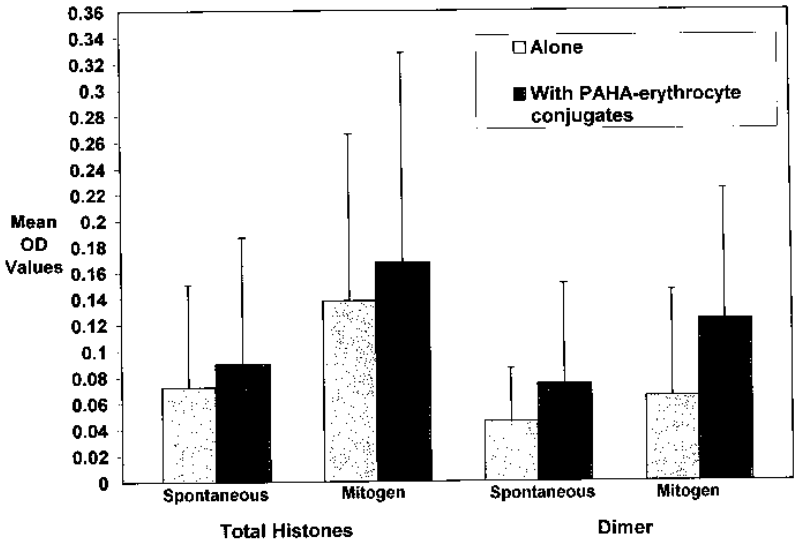

Figure 4. Effect of co-incubation of PAHA-autologous erythrocyte conjugates (PAHA-RBC) on *in vitro* production of antibodies to total histones and to the H2A-2B dimer by PBMC from patients receiving chronic procainamide. Culture conditions and antibody assays as described in Materials and Methods. Co-culture with PAHA-RBC was associated with a significant increase in spontaneous production of antibodies to total histones ($p = 0.03$) and to the H2A-2B dimer ($p = 0.009$) and in mitogen induced production of antibodies to the dimer ($p < 0.001$, Mann-Whitney).

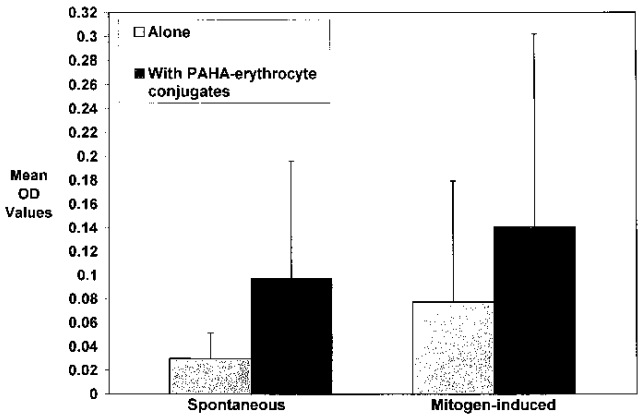

Figure 5. Effect of co-incubation of PAHA-autologous erythrocyte conjugates (PAHA-RBC) on *in vitro* production of antibodies to total histones by PBMC from controls. Co-culture with PAHA-RBC was associated with a significant increase in spontaneous production ($p < 0.001$) and mitogen induced production ($p < 0.001$) of antibodies to total histones (Mann-Whitney test).

bodies to H2A-2B, suggesting a possible immunomodulating effect by the hydroxylamine metabolite. Co-culture with these pretreated erythrocytes had a smaller effect on the production of antibodies to total histones in the unstimulated cultures, and no effect on their production in mitogen stimulated cultures. Of interest is the apparent selectivity of this effect, particularly since antibodies to the H2A-2B dimer have been associated with procainamide related autoimmunity.

In contrast, the addition of PAHA conjugated autologous erythrocytes to both unstimulated and mitogen stimulated cultures of PBMC from controls resulted in a marked increase in the production of antibodies to total histones ($p < 0.001$ by Mann-Whitney).

Relationship to the development of procainamide related lupus. During the followup period 4 patients developed procainamide related lupus, which was manifested by arthralgias, constitutional symptoms, antinuclear antibodies, and anti-histone antibodies. One patient developed symptoms within 3 weeks and another within 2 months of evaluation for this study. Mononuclear cells from both these patients, but not those of the other 2 patients, yielded spontaneous *in vitro* production of histone antibodies.

DISCUSSION

Studies have reported spontaneous *in vitro* production of IgG antibodies by B lymphocytes from patients with procainamide related lupus, and histone antibodies by PBMC from patients with idiopathic SLE[10,11]. In the latter study, *in vitro* production of histone antibodies correlated with serum histone antibody levels, suggesting that it reflected *in vivo* production. Spontaneous *in vitro* production of histone antibodies was independent of polyclonal B cell activation as measured by the total amount of immunoglobulins produced *in vitro*.

In our study there was a significant increase in spontaneous *in vitro* production of antibodies to histones by PBMC from 9 (35%) patients receiving procainamide. Of these, 7 patients had histone antibodies in their sera. Although no

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statistically significant correlation was found between *in vitro* production and histone antibodies in the respective patients' sera, spontaneous production may account for *in vivo* histone antibody production in some patients. The lack of observable spontaneous *in vitro* production by cells from 12 patients with serum histone antibodies most likely accounts for this lack of statistical correlation. However, these cell cultures may have contained histone antibodies that were not detected by the ELISA because of binding of the antibodies to histones present in the cultures. Histone antigens are expressed on cell surfaces and are also released into culture medium[11,20]. It is possible that these antigens may have bound to the histone antibodies produced *in vitro* and, by this means, interfered with their detection by the ELISA, leading to falsely negative results for some cultures.

Studies by Forrester, *et al*[10] reported a significant increase in spontaneous IgG production by B lymphocytes obtained from patients with procainamide related lupus compared with those from a control group of 6 asymptomatic patients receiving procainamide. Spontaneous production of antibodies to histones by PBMC from a large subset of SLE patients was reported to correlate with disease activity. These data suggest that spontaneous production of IgG antibodies, and in particular those with reactivity to histones, may be associated with the development of drug related lupus. We found that 2 patients whose lymphocytes showed spontaneous *in vitro* production of histone antibodies developed procainamide related lupus within 2 months of their evaluations, which would be consistent with this hypothesis. However, the other 7 patients whose lymphocytes also yielded spontaneous *in vitro* production of histone antibodies did not develop procainamide related lupus, suggesting that other factors are involved in the pathogenesis of procainamide related lupus.

Studies have suggested that the hydroxylamine metabolite of procainamide, PAHA, may be responsible for the development of procainamide related autoimmunity. Kubicka-Muranyi, *et al*[21] reported that treatment with procainamide for 16 weeks led to the appearance of PAHA related neoantigens in peritoneal cells of slow acetylator A/J mice. Recently, Goebel, *et al*[22] provided evidence for the generation of PAHA related neoantigens by peritoneal macrophages exposed to procainamide *in vitro,* and they also demonstrated that chronic *in vivo* procainamide treatment sensitizes murine T cells to PAHA related neoantigens. These studies by Gleichmann and colleagues suggest that drug modified cells may provide the hapten for T cells. Similar modification of antigen presenting cells with the formation of PAHA induced neoantigens may occur among patients receiving chronic procainamide treatment and trigger the development of an autoimmune response.

It is also possible that PAHA may act as a hapten that is rendered immunogenic by binding to a protein or large molecule. PAHA is known to readily bind to hemoglobin in erythrocytes. Enhancement of the mitogenic response of PBMC and *in vitro* Ig synthesis, as measured by reverse hemolytic plaque assay, by B lymphocytes isolated from whole blood that had been preincubated with PAHA has been reported, suggesting that PAHA can exert an immunomodulating effect on lymphocytes[7]. In our study, PBMC were cultured in the presence of PAHA conjugated autologous erythrocytes to further evaluate this possibility. The mean OD value for the patient group was statistically significantly greater for those cultures co-incubated with PAHA conjugated erythrocytes than for those cultured in their absence, suggesting an immunostimulatory effect by these conjugates. The addition of PAHA conjugated autologous erythrocytes to both unstimulated and mitogen stimulated cultures of PBMC from patients receiving procainamide resulted in significant increases in the production of antibodies to H2A-2B. The magnitude of the effect on unstimulated cultures was greater for the production of antibodies to H2A-2B than that of antibodies to total histones.

These results suggest that PAHA-erythrocyte conjugates can upregulate the immune response and that PAHA may be important in the development of procainamide related autoimmunity. These stimulatory effects may result from an antigen driven response as a result of PAHA acting as a hapten. The apparent greater effect on the *in vitro* production of antibodies to the H2A-2B dimer suggests some selectivity of the response and is of interest since the histone antibodies that are most frequently associated with procainamide related autoimmunity react with the H2A-2B dimer.

There was a significant increase in the *in vitro* production of histone antibodies by nonmitogen stimulated cells from controls that were cultured in the presence of PAHA conjugated autologous erythrocytes compared with cells that were cultured alone. A significant increase in *in vitro* production of histone antibodies was also noted for mitogen stimulated control cultures that were cultured with PAHA conjugated autologous erythrocytes compared with those cultured without PAHA conjugated autologous erythrocytes. It would be interesting to determine whether those individuals whose cells produce significant amounts of histone antibodies when co-cultured with PAHA-erythrocyte conjugates would be more at risk for development of procainamide related autoimmunity if they were prescribed this medication. Alternatively, it is possible that the expression of new or alteration of constitutive antigens on the erythrocytes, as a result of these cells being *ex vivo,* may exert an immunostimulatory effect independent of PAHA.

REFERENCES

1. Mongey A-B, Donovan-Brand R, Thomas TJ, Adams LE, Hess EV. Serologic evaluation of patients receiving procainamide. Arthritis Rheum 1992;35:219-23.
2. Rubin RL, McNally EM, Nusinow SR, Robinson CA, Tan EM. IgG

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antibodies to the histone complex H2A-H2B characterized procainamide-induced lupus. Clin Immunol Immunopathol 1985;36:49-59.
3. Totoritis MC, Tan EM, McNally EM, Rubin RL. Associations of antibody to histone complex H2A-H2B with symptomatic procainamide-induced lupus. New Eng J Med 1988;318:1431-6.
4. Ochi T, Goldings EA, Lipsky PE, Ziff M. Immunomodulatory affect of procainamide in man: Inhibition of humerous T cell activity in vitro. J Clin Invest 1983;71:36-45.
5. Bluestein HG, Redelman D, Zvaifler NJ. Procainamide lymphocyte reactions: A possible explanation for drug-induced autoimmunity. Arthritis Rheum 1981;24:1019-23.
6. Tannen RH, Cunningham-Rundles F. Inhibition of Con A mitogenesis by serum from procainamide-treated patients and patients with systemic lupus erythematosus. Immunol Comm 1982;11:33-45.
7. Adams LE, Sanders CE Jr, Budinsky RA, Donovan-Brand RJ, Hess EV. Immunomodulatory effects of procainamide metabolites: their implications in drug-related lupus. J Lab Clin Med 1989; 113:482-92.
8. Yu CL, Ziff M. Effects of long-term procainamide therapy on immunoglobulin synthesis. Arthritis Rheum 1985;28:276-84.
9. Miller KB, Salem D. Immunoregulatory abnormalities produced by procainamide. Am J Med 1982;73:487-92.
10. Forrester J, Golbus J, Brede D, Hudson J, Richardson B. B cell activation in patients with active procainamide-induced lupus. J Rheumatol 1988;15:1384-8.
11. O'Dell JR, Bizar-Schneebaum H, Kotzin BL. In vitro anti-histone antibody production by peripheral blood cells from patients with systemic lupus erythematosus. Clin Immunol Immunopathol 1988;47:343-53.
12. Kretz-Rommel A, Duncan SR, Rubin RL. Autoimmunity caused by disruption of central T cell tolerance. J Clin Invest 1997; 99:1888-96.
13. Uetrecht JP, Sweetman BJ, Woosley RL, Oates JA. Metabolism of procainamide to a hydroxylamine by rat and human hepatic microsomes. Drug Metab Dispos 1984;12:77-81.
14. Roberts SM, Adams LE, Donovan-Brand RJ, et al. Procainamide hydroxylamine lymphocyte toxicity. Evidence for participation by hemoglobin. Int J Immunopharmacol 1989;12:1-9.
15. Kiese M. Ferrihemaglobinemia: a comprehensive treatise. Cleveland: CRC Press; 1974.
16. Eyer P, Ascherl M. Reactions of para-substituted nitrosobenzenes with human hemaglobin. Biol Chem Hoppe-Seyler 1987; 368:285-94.
17. Wheeler JF, Lunte CE, Zimmer H, Heineman WR. Electrochemical investigations of immunologically reactive procainamide metabolites. J Pharmacol Biomed Anal 1990;8:143-50.
18. Wheeler JF, Adams LE, Mongey A-B, Roberts SM, Heineman WR, Hess EV. Determination of metabolically-derived nitroprocainamide in the urine of procainamide-dosed humans and rats by liquid chromatography with electrochemical detection. Drug Metabol Disposit 1991;19:691-5.
19. Budinsky RA, Roberts SM, Coats EA, Adams LE, Hess EV. The formation of procainamide hydroxylamine by rat and human liver microsomes. Drug Metab Dispos 1987;15:37-43.
20. Holers VM, Kotzin BL. Human peripheral blood monocytes display surface antigens recognized by monoclonal antinuclear antibodies. J Clin Invest 1985;76:991-8.
21. Kubicka-Muranyi M, Goebels R, Goebel C, Uetrecht JP, Gleichmann E. T lymphocytes ignore procainamide, but respond to its reactive metabolites in peritoneal cells: demonstration by the adoptive transfer popliteal node assay. Toxicol Appl Pharmacol 1993;122:88-94.
22. Goebel C, Vogel C, Wulferink M, et al. Procainamide, a drug causing lupus, induces prostaglandin H synthase-2 and formation of T cell-sensitizing drug metabolites in mouse macrophages. Chem Res Toxicol 1999;12:488-500.

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