# ROLE OF INCREASED EICOSANOID PRODUCTION IN ENHANCED IMMUNOSUPPRESSIVE ACTIVITY OF SERUM AFTER HEMOPERFUSION

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Methods of extracorporeal blood purification have been used successfully in the treatment of autoimmune bullous dermatoses, namely pemphigus vulgaris (PV) and bullous pemphigoid (BP) [2]. The therapeutic effect of these procedures is linked with the normalizing influence of hemoperfusion on lectin-dependent mononuclear proliferation [4], the interleukin cascade reaction [15], and also with the removal of pathogenic autoantibodies, immune complexes, and "blocking factors" from the adsorbed blood. One such factor has been identified in eluates from the charcoal after adsorption of blood from psoriasis patients [3]. Other investigations have shown that substances inhibiting lymphokine production also are removed from the blood [14], and serum of the adsorbed blood acquires immunosuppressive properties [6]. Despite the fact that the phenomenon of stimulation of suppressor activity of autologous serum by hemoperfusion is widely used in the treatment of various immunopathological states [1-3, 5, 6], the mechanism of this phenomenon has not yet been fully studied, for the increase in production of 2-macroglobulin and of other "acute phase" proteins, possessing an immunosuppressive effect [8], does not begin until the 15th-20th day after the procedure [2, 4].

In connection with the immunoregulatory role of eicosanoids [9, 13], the aim of this investigation was to study production of cyclooxygenase and lipoxygenase metabolites of arachidonic acid at intervals during experimental adsorption of blood from healthy donors and from patients with PV and BP.

## EXPERIMENTAL METHOD

Twelve hitherto untreated patients with PV and BP, aged from 39 to 67 years, were subjected to experimental hemoperfusion by the method described previously [1] with the UÉG-01 apparatus, using columns with type SKN charcoal hemocarbosorbent. Before the beginning of the first therapeutic hemoperfusion procedure, 500 ml of venous blood was withdrawn from the patients' blood stream, and before it was returned to the patient, it was perfused 2-10 times through a column containing 50 g of the sorbent. A similar procedure was carried out with 12 flasks (450 ml) of blood obtained from healthy blood donors aged 25-45 years at the Kiev blood transfusion station. In the course of experimental hemoperfusion, samples of adsorbed blood were taken for investigation of plasma concentrations of prostaglandins (PG) $E_2$, 6-keto-$PGF_{1\alpha}$ ($PGF_{1\alpha}$), $PGF_{2\alpha}$, thromboxane $B_2$ ($TB_2$), and leukotrienes (LT) $B_4$ and $C_4$, by radioimmunoassay, according to the instructions given by the manufacturers. In parallel tests the ability of the monocytes of the adsorbed blood to produce eicosanoids was determined. For this purpose, in the first stage the mononuclear fraction was isolated on a standard Ficoll–Verografin density gradient and the settling erythrocytes were removed by hypotonic lysis, and in the second stage, monocytes were isolated from the mononuclear suspension by centrifugation for 20 min at 800*g* on a 50% Percoll density gradient [11]. The collected cells were resuspended up to a concentration of $1 \cdot 10^6$ cells/ml in Earle's medium without phenol red, containing 10% pretested heat-inactivated (56°C, 30 min) fetal calf serum (FCS, from "Serva," West Germany), and then incubated in a $CO_2$-incubator in the presence of $10^{-5}$ M of the potassium ionophore A 23187 ("Sigma," USA). Supernatants collected after incubation of the cultures for 30 min were quickly frozen. The viability of more than

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TABLE 1. Eicosanoid Production (ng/$10^6$ cells) by Monocytes of Adsorbed Blood ($M \pm m$)

| Source of monocytes | Number of perfusions | $PGE_2$ | $PGF_{1\alpha}$ | $PGF_{2\alpha}$ | $TB_2$ | $LTB_4$ | $LTC_4$ |
|---|---|---|---|---|---|---|---|
| Donors (normal) (n = 12) | 0 | 8,2±0,3 | 0,9±0,06 | 3,2±0,2 | 9,4±1,2 | 14,3±2,6 | 8,3±0,6 |
| | 5 | 12,8±0,9 | 2,2±0,1 | 4,6±0,6 | 13,8±1,9 | 17,6±2,3 | 11,5±1,8 |
| | 10 | 18,3±0,8 | 3,7±0,4 | 9,0±1,1 | 26,6±3,3 | 32,3±5,0 | 29,3±2,4 |
| Patients with PV (n = 12) | 0 | 3,0±0,2* | 0,4±0,02* | 2,8±0,2 | 11,9±2,3 | 16,6±2,9 | 8,9±0,9 |
| | 5 | 10,1±0,8* | 1,8±0,2 | 3,3±0,7 | 15,0±1,4 | 19,9±2,5 | 13,6±1,4 |
| | 10 | 16,8±1,2 | 3,6±0,3 | 7,8±0,8 | 23,5±4,0 | 37,3±5,3 | 28,6±4,1 |
| Patients with BP (n = 11) | 0 | 4,3±0,1* | 0,5±0,07* | 4,1±0,6 | 9,6±1,0 | 15,3±2,1 | 10,2±1,5 |
| | 5 | 12,8±0,5 | 2,0±0,8 | 5,0±0,7 | 12,8±1,4 | 18,7±2,0 | 12,6±1,4 |
| | 10 | 19,9±0,9 | 3,8±0,4 | 9,3±0,9 | 25,8±3,2 | 31,6±4,8 | 27,6±3,4 |

**Legend.** Here and in Tables 2 and 3, asterisks indicate significant ($p < 0.05$) differences from corresponding values for healthy donors, in all other cases $p > 0.05$.

TABLE 2. Eicosanoid Content in Plasma of Adsorbed Blood ($M \pm m$)

| Source of plasma | No. of perfusions | $PGE_2/PGF_{2\alpha}$ | $TB_2/PGF_{1\alpha}$ | $LTB_4$, pg/ml | $LTC_4$, pg/ml |
|---|---|---|---|---|---|
| Donors (normal) (n = 12) | 0 | 3,4±0,2 | 10,6±1,2 | 226,4±18,3 | 102,7±14,3 |
| | 5 | 3,5±0,3 | 14,7±1,4 | 274,5±16,8 | 122,4±17,6 |
| | 10 | 3,8±0,5 | 13,4±1,3 | 338,4±26,2 | 156,3±10,3 |
| Patients with PV (n = 12) | 0 | 1,2±0,07* | 17,4±2,1* | 293,5±15,8* | 139,3±18,2 |
| | 5 | 2,0±0,1* | 18,3±1,6 | 304,8±19,6 | 156,3±11,9 |
| | 10 | 3,2±0,4 | 15,3±1,4 | 341,5±22,6 | 169,4±11,8 |
| Patients with BP (n = 11) | 0 | 1,5±0,09* | 13,6±0,9* | 256,3±18,3 | 124,8±10,6 |
| | 5 | 2,3±0,2* | 15,4±1,2 | 278,3±11,9 | 144,3±16,8 |
| | 10 | 3,6±0,4 | 15,3±1,7 | 317,3±20,6 | 161,7±12,3 |

90% of the tested cells was confirmed in the trypan blue test, and they were identified as monocytes (over 95%) on the basis of their staining with a 1% solution of $\alpha$-naphthyl esterase. The effect of serum from adsorbed blood on activity of the immunocompetent cells was studied by incubating them for 24 h in a $CO_2$ incubator concurrently with a suspension of mononuclears isolated from intact donor's blood on a standard Ficoll–Verografin density gradient, resuspended to a concentration of $2.5 \cdot 10^6$ cells/ml in medium RPMI 1640 ("Serva"), containing 2 mM L-glutamine, 1% of amino acids ("Gibco," Great Britain), $5 \cdot 10^{-5}$ M 2-mercaptoethanol ("Sigma"), 10 mM HEPES-buffer ("Serva"), 10% FCS, and antibiotics. The cultures were incubated in the presence of 0.5 $\mu$g/ml of phytohemagglutinin (PHA) or 1.5 $\mu$g/ml of concanavalin A (con A; Pharmacia LKB Biotechnology AB, Sweden). $^3$H-Thymidine (15 Ci/mmole, from "Amersham," Great Britain), was added 6 h before the end of culture. The results were expressed as an index of suppression (IS), which was calculated by the equation:

$$IS = \frac{\text{Av. (cpm) of 3 paralle samples containing serum from adsorbed blood}}{\text{Av. (cpm) of 3 parallel control samples (without serum)}} \cdot 100.$$

## EXPERIMENTAL RESULTS

Filtration of the blood through a column with the hemocarbosorbent had a stimulating effect on eicosanoid production by monocytes of the healthy donors and patients with autoimmune dermatoses (Table 1). Ability of the monocytes of patients with PV and BP to produce $PGE_2$ and $PGF_{1\alpha}$, which initially was depressed, was restored after five perfusions. Ratios of concentrations of cyclooxygenase metabolites of arachidonic acid, which, according to many workers, can provide an objective evaluation of the pathophysiological effect of a change in the content of these biologically active substances, with a mutually opposite action [7], and also plasma levels of lipooxygenase metabolites in dermatologic patients, were finally restored to normal after 10 perfusions (Table 2). First, the results add to existing information on the stimulating effect of hemoperfusion on activity of immunocompetent cells, and second, they shed light on one of the reasons why serum from adsorbed blood acquires immunosuppressive properties (Table 3). The increase in suppressor activity, it is important to note, was due to increased production, in the course of experimental hemoperfusion, of $PGE_2$, which has a marked immunosuppressive action [13]. Meanwhile, the higher values of IS

TABLE 3. IS (in %) of Sera from Adsorbed Blood ($M \pm m$)

| Number of perfusions | Source of serum | | |
|---|---|---|---|
| | donors (normal) (n = 12) | patients PV (n = 12) | patients with BP (n = 11) |
| 0 | 9,2±0,8 | 4,6±0,6* | 5,2±0,4* |
| 2 | 10,4±0,9 | 6,9±0,7* | 9,2±1,2 |
| 4 | 14,4±1,5 | 13,8±1,9 | 15,8±1,3 |
| 6 | 17,6±1,4 | 19,8±2,0 | 18,2±1,6 |
| 8 | 19,3±1,7 | 25,3±2,0* | 21,4±2,0 |
| 10 | 20,3±2,2 | 38,3±4,5* | 29,6±3,8* |

in the serum of patients with PV and BP compared with that from healthy blood donors are evidence of the existence of a "reserve" of humoral immunosuppressive factors in these autoimmune dermatoses we are discussing.

Considering the information on a deficiency of interleukin inhibitors in the blood serum of patients with autoimmune diseases [10], on their fixation on membranes of immunocompetent cells [12], and also on the absorption of these substances by charcoal during experimental hemoperfusion [15], it can be tentatively suggested that as a result of contact between the blood and the sorbent, a larger quantity of "inhibitory factors" convert from the bound into the free state in autoimmune patients than in the case of healthy blood donors.

The results of this investigation thus confirm the fact established previously that eicosanoid production is enhanced after contact between monocytes and foreign material, and they indicate that the increase in immunosuppressive activity of the serum from adsorbed healthy human blood is directly dependent on elevation of the plasma PGE concentrations. Treatment of patients with PV and BP by hemoperfusion restores dynamic equilibrium in arachidonic acid metabolism, which initially is shifted toward increased LT production, and it also leads to an increase in immunosuppressive activity of the serum, due both to an increase in the eicosanoid concentration and to the appearance of other, as yet unidentified, "inhibitory factors" in the circulation.

## LITERATURE CITED


1. S. A. Grando, B. T. Glukhen'kii, A. B. Romanenko, et al., Vestn. Dermatol., No. 7, 6 (1988).
2. S. A. Grando, B. T. Glukhen'kii, N. S. Kutsenko, et al., Staggered Differential Combination Treatment of Patients with Pemphigus Vulgaris [in Russian], Kiev (1987).
3. N. G. Korotkii and D. D. Petrunin, Vestn. Dermatol., No. 6, 11 (1983).
4. Yu. M. Lopukhin, M. N. Molodenkov, N. G. Evseev, et al., Vestn. Dermatol., No. 1, 8 (1980).
5. Yu. M. Lopukhin and M. N. Molodenkov, Hemoperfusion [in Russian], Moscow (1985).
6. M. N. Molodenkov, A. N. Cheredeev, V. F. Lyakhov, and G. Ya. Sharapova, Immunologiya, No. 6, 65 (1985).
7. J. Musil, Principles of Biochemistry of Pathological Processes [Russian translation], Moscow (1985).
8. E. W. Ades, A. Hinson, C. Chapnis-Cellier, and P. Arnaud, Scand. J. Immunol., **15**, 109 (1982).
9. M. A. Bray, Agents and Actions, **19**, 87 (1986).
10. J. Y. Djeu, T. Kasahara, J. E. Balow, and G. C. Tsokos, Clin. Exp. Immunol., **65**, 279 (1986).
11. N. R. Ferreri, W. C. Howland, and H. L. Spiegelberg, J. Immunol., **136**, 4188 (1986).
12. T. Fukushima, K. Kobayashi, T. Kasama, et al., Int. Arch. Allergy, **84**, 135 (1987).
13. J. S. Goodwin, J. L. Ceuppens, and N. Gualde, Adv. Inflammat. Res., **7**, 79 (1984).
14. S. A. Grando and B. T. Glukhen'kii (B. T. Glukhenky), Dermatology in the Developing World, Oxford (1988), pp. 68-69.
15. S. A. Grando, B. T. Glukhen'kii (B. T. Glukhenky), G. N. Drannik, et al., Immunology, **66**, 138 (1989).