The Influence of Antiserum to Human Erythropoietin on the Production of Hemoglobin C in Goats[1] (34928)

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Urinary extracts with relatively high erythropoietic activity have been used to stimulate the production of the hemoglobin β^C ($Hb_\beta C$) chain which is the product of a dormant structural gene in certain sheep and in goats (1–4). Consequently, an erythropoietic stimulating factor (ESF) has been implicated with the activation of the gene. However, because of the impurity of ESF extracts, the involvement of more than one factor in this process should be considered. The experiments to be described here were designed to see if antisera with ESF-neutralizing activity would (partially) prevent the production of Hb-C in goats made anemic with phenylhydrazine (PH) and in goats injected with a urinary extract which had high ESF activity prior to incubation with a rabbit anti-ESF serum.

Animals. Five goats were each made anemic with a single intravenous injection of 20 mg PH per kg body weight. One day later three goats were injected similarly with one of two rabbit anti-human ESF sera (1.35–1.65 ml/kg). One tenth milliliter of one of the antisera neutralized 0.21 ± 0.01 (standard error of mean) IU ESF (10 mice) whereas the other neutralized 0.11 ± 0.02 IU ESF (10 mice). One control goat received no serum, whereas a second one received a non-neutralizing anti-ESF serum (1.2 ml/kg).

Twin goats (Nos. 3 and 4, 8 kg and 8 months old) were injected intraperitoneally with a mixture of rabbit serum and human ESF extract, whereas one control goat was injected with only normal rabbit serum (4 ml/kg). Goat 3 received 450 IU human ESF in 4 ml of normal rabbit serum per kg. The ESF extract was prepared from the urine of a patient with paroxysmal nocturnal hemoglobinuria by chromatography on DEAE–cellulose (5). The serum potentiated the ESF activity by about 15%, so that this goat received some 500 IU ESF per kg. Goat 4 was injected with 450 IU of the same ESF/kg, but this material was dissolved in 4 ml of a potent ESF-neutralizing antiserum. The two ESF–serum mixtures were incubated for 30 min at 37° and centrifuged for 20 min at 1570*g* prior to injection of the supernatant fluid. There was a considerable precipitate in the mixture of the ESF extract with the anti-human ESF serum, whereas the precipitate in the mixture of the ESF with the normal serum was negligible. The antiserum in which the human ESF was dissolved neutralized all the ESF activity, and the immunopotency of the supernatant was still considerable [0.001 and 0.002 ml neutralized 0.12 and 0.28 ± 0.03 IU of human ESF respectively (10 mice), whereas the same amount of antiserum also neutralized 0.17 and 0.26 ± 0.02 IU of goat ESF (10 mice)]. The goats were starved the day before, the day during, and the day after the injections to subdue the production of endogenous erythropoietic stimulating factor(s).

Preparation of ESF antisera. The ESF antisera were produced as described by Lange, Gardner, Wright, and Gallagher (6). The antigen was in an ESF extract of the urine from a patient with anemia secondary to multiple myeloma. The immunopotency of the antiserum was determined from the neutralization of the activity of a known amount of human or of goat ESF during incubation in 0.1 and 0.2 ml of the antiserum (or some specified dilution of the serum when indicated). The goat ESF was in serum from goats made anemic by bleeding (7).

Additional methods. The procedure for the

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determination of ESF activity was a modification of the technique by Pavlovic-Kentera *et al.* (8) and Lewis *et al.* (9, 10). The ESF activity, as measured with posthypoxic polycythemic mice, is expressed as international standard B units (11), which were obtained from a dose-response curve (12).

The relative amounts of hemoglobin A and C were determined by chromatography on columns of DEAE–Sephadex (13, 14). Hematologic analyses which included total Hb (g/100 ml), PCV (%), RBC (10^6/mm^3), and reticulocyte (%) counts were made with standard laboratory techniques (15).

Results. The injection of 20 mg PH/Kg resulted in decreases in the PCV values of 12–20% in all goats. The maximal decrease was observed 12 days after the injections. Hb-C increased maximally from about 10% to 65–90% in all goats. No appreciable differences between the controls and the goats treated with rabbit anti-ESF serum were seen. The same was probably true for the ESF activity of the sera of these goats, although the maximal ESF values were 0.21–0.36 IU/ml serum in control goats and 0.11–0.18 IU/ml serum in the goats receiving anti-ESF serum.

Goats 3 and 4 tolerated the injection of the ESF–rabbit serum mixtures without any apparent side effects. A slight decrease in the PCV values was observed in both animals (Fig. 1) and in the control goat receiving only rabbit serum. The level of total Hb and number of red cells corresponded closely with the variations in the PCV values. The level of Hb-C in goat 3 increased from 7 to 34% in 50 days, whereafter a steady decrease occurred. The level of Hb-C in goat 4 fluctuated between 1 and 8% during the entire experiment. The ESF activity of the serum in both goats was measured in polycythemic mice and apparently increased slightly during the first 25 days; reticulocytosis was absent. The two goats gained about 4 kg during the experimental period.

Discussion. The ESF-neutralizing antibodies in goats which were made anemic with PH possibly neutralized some of the ESF activity in these animals. The small (if any) decrease in ESF activity was not accompanied with an appreciable decrease in the percentage of Hb-C.

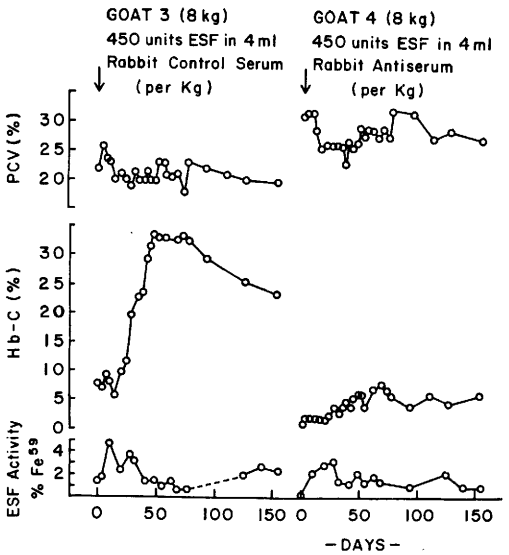

FIG. 1. The ESF–rabbit serum mixture indicated above was incubated for 30 min at 37°, centrifuged for 20 min, and the supernatant fluids were injected intraperitoneally into goats 3 and 4. The ESF in the rabbit antiserum was completely neutralized.

The effect of ESF-antiserum on the activity of ESF was marked, since it greatly suppressed the production of Hb-C in goat 4 which received the mixture of the antiserum and ESF extract of high potency. The maximal Hb-C production in goat 4 was, indeed, much lower than that in goat 3 which received the same ESF extract but in normal rabbit serum.

The slight decrease in the PCV values observed in the three goats was possibly caused by a slight hemolysis which followed the injection of the ESF extract and rabbit serum mixture and by a change in extracellular plasma volume brought about by the injection of 4 ml rabbit serum/kilogram. This effect is rather surprising because one would expect an erythrocytosis of considerable magnitude with increased, rather than decreased, PCV values similarly as observed previously in a goat injected with a comparable amount of ESF(s) (4). A small part of the increased production of Hb-C in goat 3, comparable to the amount seen in goat 4, was likely due to the slight decrease in the PCV values.

The study by Reisman *et al.* showed that 50% of the ESF which was obtained from rabbits made anemic with PH and injected into normal rats disappeared from the serum during the first hour and the remainder exponentially in a matter of a few hours (16). It seems then that the observed ESF activity in goats 3 and 4 probably represents a day by day endogenous production of goat ESF. The similar levels of ESF activity in both animals, in spite of the high immunopotency of the anti-human ESF serum injected into goat 4, suggests the presence of a different ESF antigen. Two ESF antigens, different in that one produced neutralizing and the other non-neutralizing antibodies, have been isolated from similar extracts (17–19).

Several explanations have been presented for the biologic switch of the $Hb_{\beta}A \rightleftharpoons Hb_{\beta}C$ structural genes (4). The current study strongly supports the hypothesis that humoral factor(s) are involved in the activation (or inhibition) of this mechanism. Our data again implicate erythropoietic-stimulating factors, but do not add much to our understanding of the nature of these humoral factors. The ESF extracts contain at least three different erythropoietic factors (12, 17, 19, 20), and the antiserum responsible for suppressing the production of Hb-C in goat 4 possibly contained antibodies to all three.

Summary. The production of Hb-C was studied, with and without the presence of antibodies to ESF(s), in goats made anemic by injections of phenylhydrazine. Although the ESF(s) activity was possibly slightly decreased by the relatively weak anti-ESF serum, the production of Hb-C was not altered appreciably.

Twin goats were injected with ESF(s) dissolved in rabbit serum without and with potent neutralizing antibodies to ESF. There was a marked increase in Hb-C production in the goat receiving the ESF in normal serum, but the Hb-C in the goat receiving antiserum with the ESF activity neutralized was minimal.

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