# MICROBIOLOGY AND IMMUNOLOGY

# Induction of Prolactin Receptors in Cholangiocytes of Male and Female Rats after Ligation of the Common Bile Duct

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Intense expression of prolactin receptors in cholangiocyte nuclei, nuclear membranes, and cytoplasm was demonstrated by the indirect immunoperoxidase method in intact and gonadectomied male and female rats after ligation of the common bile duct. The intensity of staining increased during cholangiocyte proliferative response to the intervention and, in contrast to hepatocytes, did not depend on animal sex and the presence of the gonads.

**Key Words:** *prolactin receptors; immunohistochemistry; rat cholangiocytes; proliferation; sex hormones*

Prolactin contributes to the regulation of proliferative response. In hepatocytes prolactin is a permanent regulator of numerous metabolic processes. Hepatocytes are characterized by a high sex-dependent content of prolactin receptors (PR) [1,2,4,14,15]. The probability of prolactin effect on cholangiocytes and its role in the regulation of proliferative and metabolic activity of these cells is unknown, in contrast to its effect on hepatocytes.

Previously, we revealed transitory expression of PR in male and female rat cholangiocytes during the period preceding the onset of sexual maturating [1]. This period of ontogenesis is characterized by high proliferative activity of liver cells. Therefore, we suggested that PR are exposed in cholangiocytes only during their intensive hyperplasia. This study was undertaken to verify this hypothesis by inducing cholangiocyte proliferation in response to ligation of the common bile duct (CBD).

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## MATERIALS AND METHODS

Adult male and female albino rats of mixed population, intact and 3 weeks after removal of the gonads, were used. The common bile duct was ligated by the standard method [3]; liver tissue was obtained 2, 7, and 14 days after the intervention. Material from 3-4 animals was analyzed for each group. Sham-operated intact or gonadectomied animals served as controls.

The localization of PR in liver cells was determined by the indirect immunoperoxidase method [14]. Anti-PR antibodies — murine monoclonal antibodies (clone U6) to a site of rat extracellular PR domain other than the hormone-binding center, were a generous gift of Dr. P. Kelly (France) [10]. The specimens were fixed in 4% paraform for 18-20 h at 4°C and embedded in paraplast. After pretreatment with 10 mM sodium periodiate and 0.01% sodium boron hydride, 3-μ-thick sections were incubated with monoclonal antibodies (0.1 mg/ml IgG fraction of ascitic fluid) in 0.05 M Tris-HCl buffer, pH 7.6, for 18-20 h at 4°C. Control sections were incubated under the same conditions with 0.05 M Tris-HCl

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buffer or murine IgG solution (0.1 mg/ml) in the same buffer. Bridge and development antibodies were, respectively, rabbit antimurine antiserum (1:10) and peroxidase-labeled asinine antirabbit antibodies (1:100) (Institute of Epidemiology and Microbiology) exhausted with rat serum. Bridge and development antibodies were incubated with experimental and control sections for 30 min at room temperature. After each stage of treatment, the sections were washed three times in the buffer for 5 min. Chromogen was diaminobenzene. Some sections were counterstained with hematoxylin.

## RESULTS

The intensity of PR espression in cholangiocytes of adult female rats gradually increased in response to ligation of CBD and reached the maximum 14 days after the operation (Fig. 1). By this time, the number of bile ducts in the periportal zone increased from 1-2 to 7-15, and a slight peribiliary inflammation and fibrosis developed. A similar time course of PR expression was observed in cholangiocytes of adult males after ligation of CBD.

Figure 2 shows that the expression of PR in male rat cholangiocytes 14 days after CBD ligation was higher than in intact males. Due to increased size of cholangiocyte during cellular proliferative response to the intervention [3,13], we identified a subcellular distribution of PR. PR-positive staining, more intense than the basic level, was observed in cholangiocyte nuclei, nuclear membranes, and cytoplasm (Fig. 2). Staining with hematoxylin showed a high number of actively dividing cells in the bile ducts during this period after operation (Fig. 2).

Fourteen days after CBD ligation, the expression of PR in hepatocytes of adult females was essentially higher than in males, whereas in hepatocytes of gonadectomied males and females its level was intermediate (Fig. 3). This corresponds to regularities of PR manifestation in hepatocytes of the respective groups of animals with intact bile ducts [14]. In

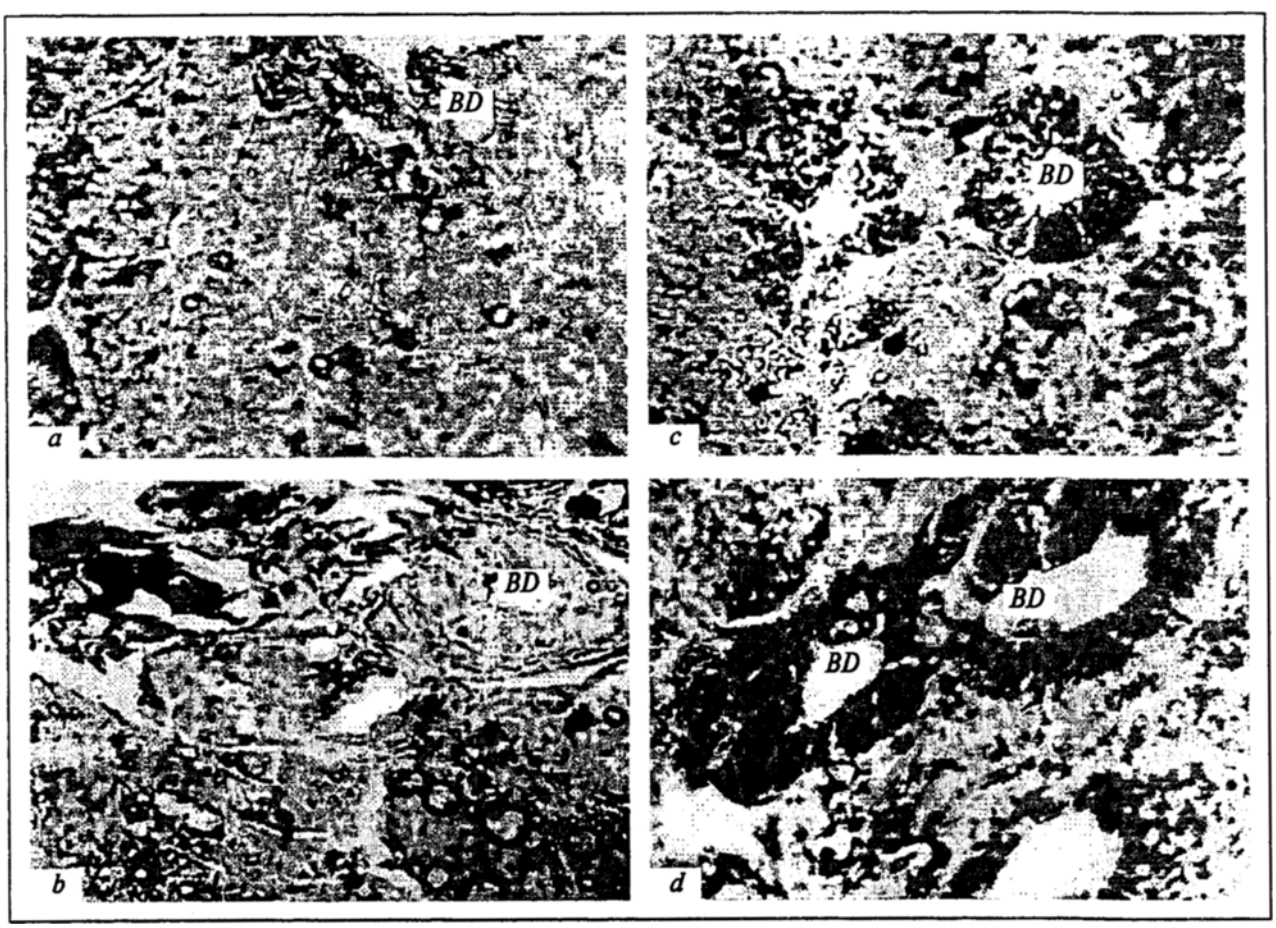


**Fig. 1.** Time course of prolactin receptor expression in female rat cholangiocytes during their proliferative response to ligation of the common bile duct. ×787.5. Immunoperoxidase identification of prolactin receptors in cells of periportal zone of hepatic lobule of intact female (*a*) and 2 (*b*), 6 (*c*), and 14 (*d*) days after ligation of the common bile duct. Here and in Figs. 2 and 3: *BD*) bile duct.

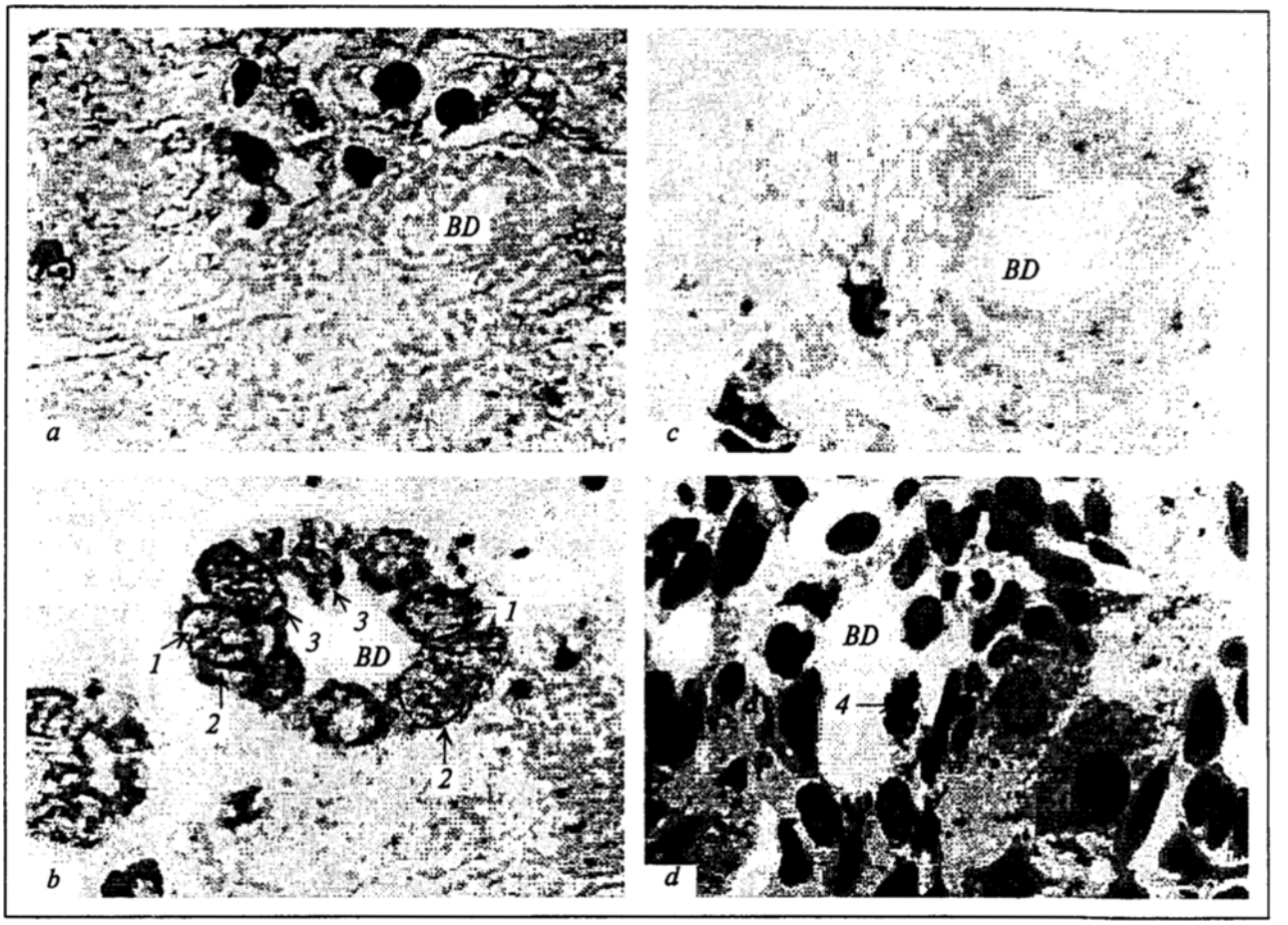


Fig. 2. Subcellular distribution of prolactin receptors in cholangiocytes of male rat 14 days after ligation of the common bile duct. ×787.5. Immunoperoxidase identification of prolactin receptors in cells of periportal zone of hepatic lobule of intact male (*a*) and 14 days after ligation of common bile duct (*b*). Immunoperoxidase staining of control sections without (*c*) and with hematoxylin staining (*d*). *1*) positive staining of nuclei; 2) nuclear membranes; *3*) cytoplasm; *4*) nuclei of mitotically active cholangiocytes.

contrast to hepatocyte PR, PR expression in cholangiocytes after ligation of CBD was equally high in males and females and did not depend on the presence of the gonads (Fig. 3).

Thus, our data confirm the hypothesis on increased expression of PR in cholangiocytes during their intense hyperplasia occurring for 1-4 weeks after ligation of CBD [3,9,12,13]. The role of increased sensitivity of bile duct cells to prolactin during this period is not clear. After ligation of CBD, cholangiocytes acquire a higher capacity for expression of proteins somehow related to cell proliferation (transforming growth factor-β, receptors of insulin-like factor II/mannose-6-phosphate) and increased sensitivity to factors stimulating their functional activity such as regulation of water-electrolyte composition of the bile [3,12]. Presumably, prolactin is necessary for maintaining high mitotic activity of cholangiocytes after the operation, since this hormone stimulates cell proliferation in many tissues [4,15]. Increased sensitivity of cholangiocytes to prolactin after the intervention may be explained by the need in additional stimulation of their secretory activity under the effect of this hormone. This hypothesis is in line with the data on the essential increase in the sensitivity of cholangiocytes of operated animals to secretin, another stimulator of this process [3,12], and with reports about stimulating effect of prolactin on water and electrolyte transport through the epithelium of other gastrointestinal compartments with high levels of PR [7,8].

Similarly to PR expression in cholangiocytes of young animals [1], PR compartmentalization in proliferating cells of bile ducts differs from its subcellular localization in hepatocytes of intact and regenerating liver [1,14] and, as shown in this study, in hepatocytes after ligation of CBD. The main difference is the predominant manifestation of PR in nuclear structures. Since there are at least two forms of PR with different ways of signal transduction, presentation in different tissues, and capacity to internalization [5,11], it can be suggested that the type of

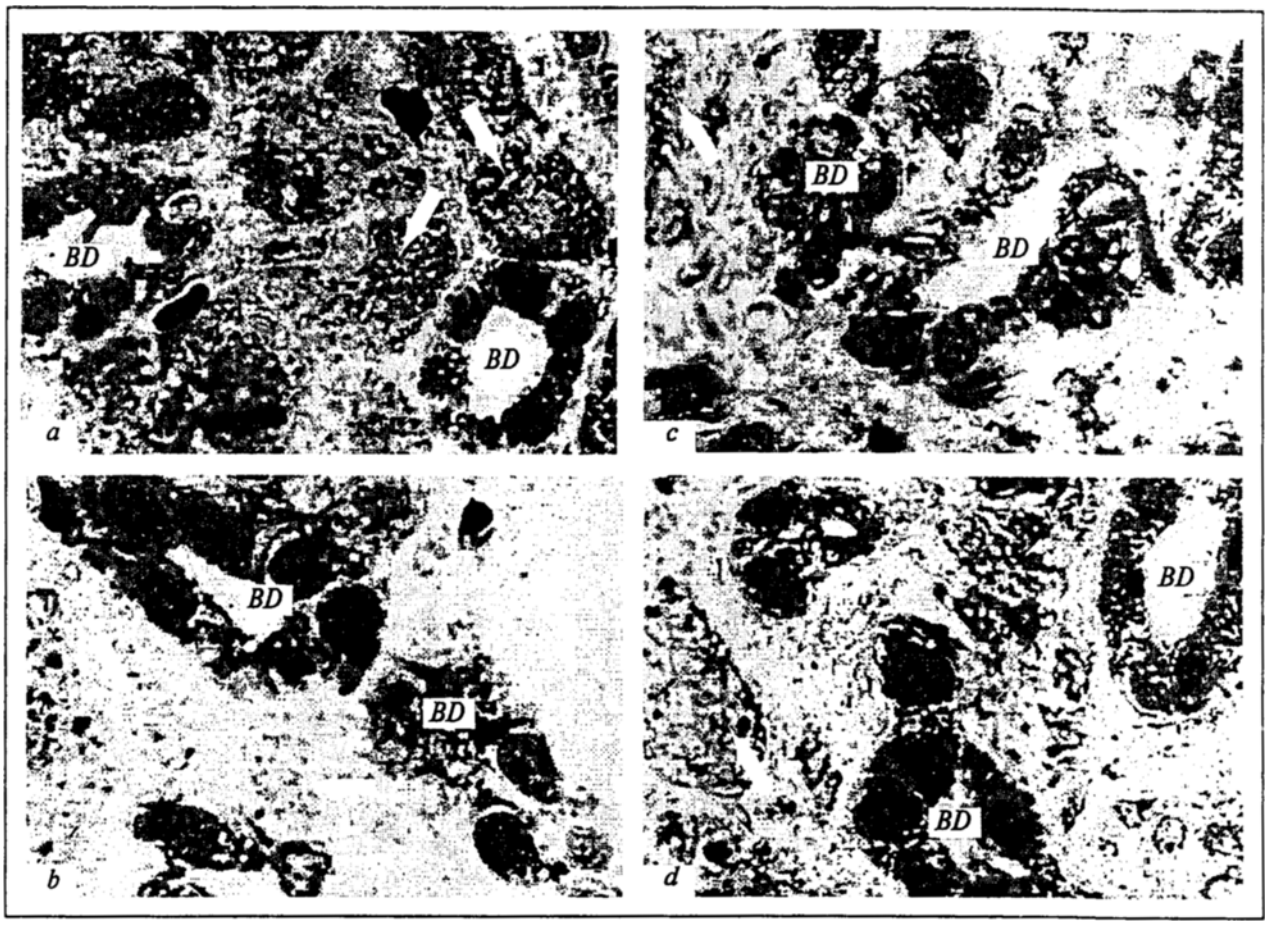


Fig. 3. Intensity of expression of prolactin receptors in cholangiocytes and hepatocytes of rats with different levels of sex hormones 14 days after ligation of the common bile duct. ×787.5. Immunoperoxidase identification of prolactin receptors in cells of periportal zone of hepatic lobule of female (*a*) and male (*b*) with intact gonads, gonadectomied female (*c*) and male (*d*) rats 14 days after ligation of common bile duct. Arrows indicate PR-positive staining of hepatocytes.

compartmentalization depends on the form of PR. Different forms of these receptors can predominate in different types of liver cells.

We showed that after ligation of CBD the intensity of PR expression increases similarly in cholangiocytes of male and female adult rats, despite essential differences in the levels of circulating estrogens and androgens which can be regarded as positive and negative regulators of PR level in rat hepatocytes [1,14]. These data and the absence of the effect of gonadectomy implies that other hormones, specifically prolactin, another positive regulator of PR expression in hepatocytes, can regulate the intensity of PR expression in proliferating cholangiocytes [2]. However, the differences in the reaction of PR of actively proliferating hepatocytes and of hepatocytes to androgens, estrogens, and proliferative stimulus, suggest that the expression of PR in these two cell types is regulated by different promoters [6].

Thus, we have shown that intense expression of PR independent of sex and levels of female and male sex steroids occurs in the course of proliferative response of cholangiocytes to ligation of CBD.

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# There Is No Relationship between the Activities of Mono- and Polyclonal Antirhesus Immunoglobulins *In vitro* and *In vivo*

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Monoclonal anti-D antibodies induce lysis of rhesus-positive erythrocytes in the antibody-dependent cytolysis test with blood mononuclear cells, the activity of the best antibodies being 10-15 times higher than of polyclonal IgG. The activities of anti-D antibodies in the cytolysis test with monocytes and lymphocytes varied: only one monoclonal antibody was highly active in both tests, whereas polyclonal IgG caused no lysis of erythrocytes in the cytolysis test with monocytes but was active in the same test with lymphocytes. Monoclonal antibodies intravenously administered to rhesus-negative recipients after transfusion of rhesus-positive erythrocytes stimulated their clearance, but its rate was slower than with polyclonal antibodies. Erythrocytes sensitized *in vitro* with monoclonal antibodies were completely removed from circulation within 3 h. It is not clear the results of which of the studied tests correlate with the capacity of anti-D immunoglobulins to block rhesus sensitization.

**Key Words:** *antirhesus immunoglobulin; monoclonal antibodies; antibody-dependent cytolysis; erythrocyte clearance; hemolytic disease of newborns*

Passive immunization of rhesus-negative women with antirhesus immunoglobulin during the first days postpartum effectively prevents rhesus sensitization and drastically decreases the incidence of hemolytic diseases of newborns. Although the prophylaxis has been carried out all over the world for about 30 years, the mechanism of immunosuppression is not clear [3]. It is believed that the effect is caused by accelerated clearance of erythrocytes (ER) of a rhesus-positive fetus from maternal circulation due to interactions between cells expressing Fcg-receptors with Fc-fragments of IgG antibodies on sensitized ER. *In vitro* target cells are destroyed by monocytes carrying FcgI-receptor and by K-cells with FcgIII-receptor by means of antibody-dependent cytolysis (ADC) [7,9]. The ADC test is considered as an accurate model of the mechanism of ER destruction in the organism [2,4].

Creation of monoclonal antirhesus immunoglobulin [1] involved study of its functional activity in comparison with the currently used polyclonal preparations whose efficacy is proven. However, lythic activity of immunoglobulins in variants of ADC did not correlate with their capacity to accelerate elimination of rhesus-positive ER from the circulation of rhesus-negative subjects.

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