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## Mumps Virus Decreases Testosterone Production and Gamma Interferon-Induced Protein 10 Secretion by Human Leydig Cells

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**Mumps virus is responsible for sterility. Here, we show that the mumps virus infects Leydig cells in vitro and totally inhibits testosterone secretion and that ribavirin in mumps virus-infected Leydig cell cultures completely restores testosterone production. Moreover, we show that gamma interferon-induced protein 10 (IP-10) is highly expressed by mumps virus-infected Leydig cells and that ribavirin does not block IP-10 production.**

Mumps virus causes testicular disorders in humans (6). Inflammation of the testis leads to hypofertility and testicular atrophy, which causes sterility that is usually transient but permanent in rare cases (8). Mumps virus replicates in the testes of mice (14), and a morphological study of the testes of patients infected with mumps virus showed degeneration of the seminiferous epithelium (4). The disruption of spermatogenesis may be a consequence of a drop in testosterone secretion by Leydig cells. Aiman et al. (1) have shown that Leydig cells are damaged by mumps virus. Morphological observation of mumps virus-infected testis showed lymphocyte infiltration of the interstitial space (10), where chemokines, which are cytokines involved in the chemoattraction of many different subtypes of leukocytes (2), may be secreted. In the present study, human Leydig cells were isolated from the testes of patients who underwent orchidectomy, as previously described (5, 7). We characterized this cell type by measuring the expression of the luteinizing hormone receptor (LHR) at the surfaces of Leydig cells using flow cytometry analysis (fluorescence-activated cell sorting). Fluorescence-activated cell sorting with a monoclonal anti-LHR antibody (2.5  $\mu\text{g/ml}$ , clone LHR29; a gift of E. Milgrom [9]) and an appropriate mouse immunoglobulin G1 (IgG1) isotype control showed that more than 87% of the cells expressed LHR (Fig. 1A). Moreover, the Leydig cell culture medium was assayed for testosterone with a competitive-radioimmunoassay (RIA) kit (Immunotech, Marseille, France) that uses <sup>125</sup>I-labeled testosterone as a tracer. Our data demonstrate that the hormone was produced for at least 6 days of culture (Fig. 1B). Then, primary Leydig cell cultures and cultures of the VERO cell line, used as a positive control, were performed in the presence and absence of 10<sup>4</sup> PFU of mumps virus (Vit-MA strain isolated from a patient at the Centre Hospitalier Regional Universitaire Hospital of Reims, France) per ml. After 5 days, the VERO cells formed syncytia in which the virus was detected by immunostaining

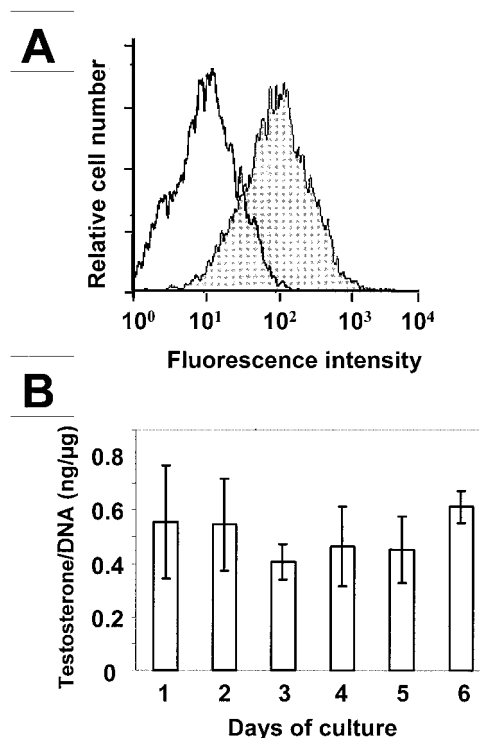


FIG. 1. Characterization of LHR expression and testosterone production by human Leydig cells. (A) Human Leydig cells were isolated from whole testis and cultured in the presence of 100 mU of human chorionic gonadotropin per ml for 4 days. The expression of LHR was detected by flow cytometry and indirect staining. Cells were first treated with 2.5  $\mu\text{g}$  of mouse anti-human LHR monoclonal antibody (LHR29) or with mouse IgG1 as a control. Then, cells were stained with phycoerythrin-conjugated goat anti-mouse IgG and examined for fluorescence by flow cytometry. The results presented are representative of three experiments performed with three different patients. (B) Human Leydig cells were cultured for 6 days in the presence of 100 mU of human CG/ml. Cell supernatants were collected every day, and testosterone concentrations were measured by a specific RIA. The results presented are representative of six experiments performed with six different patients.

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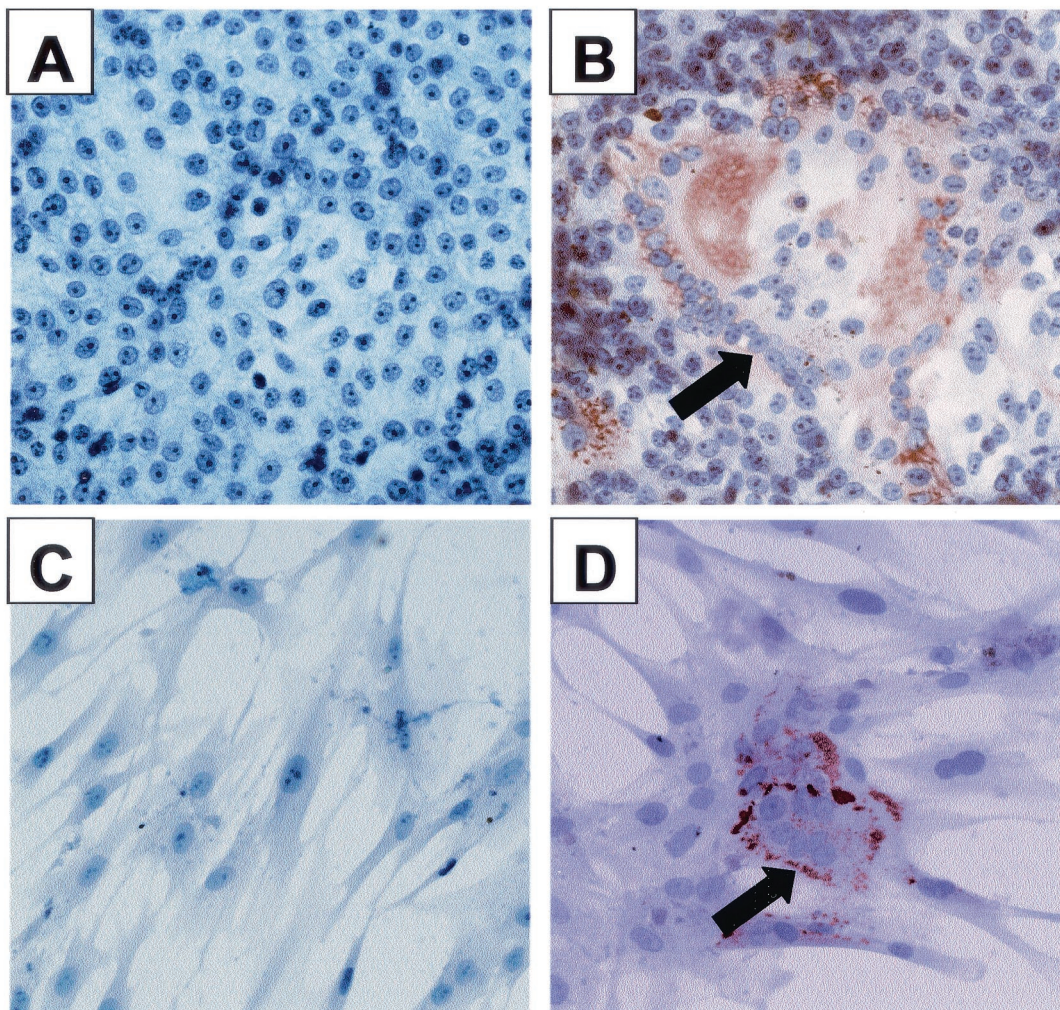


FIG. 2. Immunodetection of mumps virus in human Leydig cells and Vero cells. Vero cells (A and B) and human Leydig cells (C and D) were cultured in the absence (A and C) or in the presence (B and D) of  $10^4$  PFU of mumps virus per ml for 4 days. Immunocytochemical staining was performed with a commercial antibody specific to mumps virus as described in the text. The arrows indicate syncytium formation.

with a commercial monoclonal antibody (clone 75; 100-fold dilutions; ARGENE/BIOSOFT, Varilhes, France) (Fig. 2B). Similarly, infected human Leydig cells displayed the formation of syncytia, in which the mumps virus was detected (Fig. 2D). The replication of mumps virus was investigated. Leydig cell culture medium was harvested 6 days after infection and tested for its ability to infect the Vero cell line. Medium from Leydig cells not infected with mumps virus did not lyse the Vero cells. In contrast, the medium from Leydig cells infected with mumps virus was able to infect the Vero cell cultures even when it was diluted 1:10,000 (data not shown). The presence of ribavirin, an inhibitor of mumps virus replication, did not prevent Vero cell line lysis due to the mumps virus-infected Leydig cell culture medium, but the culture medium lysed the Vero cells only when it was diluted 1:100 or less (data not shown). Thus, we demonstrate the *in vitro* replication of mumps virus in Leydig cells. The measurement of testosterone levels in Leydig cell-conditioned medium in the presence and absence of virus and with and without ribavirin showed that

Leydig cells produced testosterone in the absence of the virus and in the presence of ribavirin alone but that testosterone production was totally inhibited by mumps virus in the absence of ribavirin at all time points tested (Fig. 3). The presence of ribavirin in the mumps virus-infected Leydig cell cultures consistently and completely restored testosterone production (Fig. 3). Aiman et al. (1) have previously shown that mumps virus orchitis is associated with reduced levels of plasma testosterone. Here, we confirm that, *in vitro*, the mumps virus can substantially impair testosterone production. This mechanism of action of the virus on steroidogenesis is unknown. However, ribavirin restored testosterone production in mumps virus-infected Leydig cells, indicating that the viral cycle, and not only viral contact with the plasma membrane or viral penetration of the cell host, is required for testosterone inhibition. Finally, to analyze leukocyte infiltration observed in cases of testicular inflammation of infectious origin, we screened, using Northern blot analysis, the expression of the chemokine transcripts RANTES (regulated on activation, normal T-cell expressed

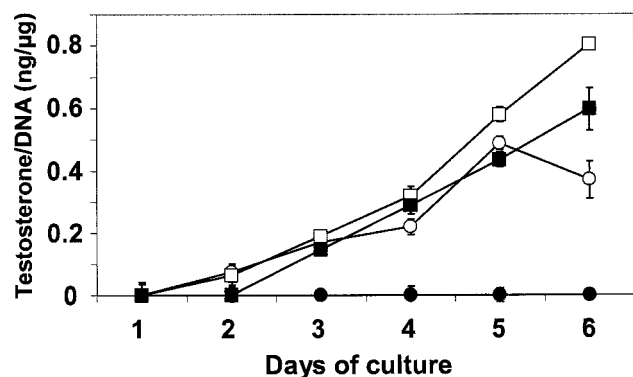


FIG. 3. Kinetics of testosterone production in a conditioned medium of human Leydig cells exposed to mumps virus. Human Leydig cells were cultured in culture medium alone (○), in the presence of 25  $\mu\text{g}$  of ribavirin per ml (□), in the presence of  $10^4$  PFU of mumps virus per ml (●), and in the presence of  $10^4$  PFU of mumps virus per ml plus 25  $\mu\text{g}$  of ribavirin per ml (■). Testosterone concentrations in conditioned medium were determined by the specific RIA. The results presented are representative of three experiments performed with different batches of isolated cells.

and secreted), growth-related oncogen  $\alpha$ , monocyte chemoattractant protein 1, and gamma interferon (IFN- $\gamma$ )-induced protein 10 (IP-10) in mumps virus-infected Leydig cells. Uninfected Leydig cells were used as negative controls. The only chemokine transcript detected in infected cells was IP-10 (data not shown). We used a specific enzyme-linked immunosorbent assay (ELISA) to determine IP-10 protein levels (Tebu-Bio, Le Perray-en-Yvelines, France) in cell culture supernatants collected daily. The level of IP-10 production was very low in the absence of virus or the presence of ribavirin alone. In contrast, IP-10 was abundant in the medium as early as 1 day postinfection with  $10^4$  PFU of mumps virus per ml with or without ribavirin (Fig. 4). IP-10 secretion was highest on days 2 and day 3 and then declined to control levels by day 5. IP-10 production by various cell types is induced by IFN (13, 15), so we assayed IFN- $\alpha$ , IFN- $\beta$ , and IFN- $\gamma$  concentrations in Leydig cell-conditioned medium. Under all conditions tested, the concentrations of the different IFN types investigated were lower than the sensitivity threshold concentration of the ELISA used. It was thus concluded that mumps virus did not induce IFN expression by Leydig cells (data not shown). Thus, we demonstrate that Leydig cells can produce a chemoattractant molecule, IP-10, which may be involved in the etiopathology of a testicular viral infection, such as mumps virus infection, by attracting lymphocytes to the interstitial compartment. It also shows that, in our model, IP-10 expression is not dependent on IFN. Similarly, glioblastoma cells exposed to the measles virus, another member of the *Paramyxoviridae* family, express IP-10 independently of IFN (11). It is possible that a component of the mumps virus capsid directly induces IP-10 expression, as was previously shown for adenovirus (3). Ribavirin did not block IP-10 production by mumps virus-infected Leydig cells, but viral replication was inhibited, indicating that the mumps virus replication process is not required for IP-10 production. This finding supports the view that components of the virus membrane induce IP-10 production, as was observed for RANTES production by measles virus-infected astrocytes (12).

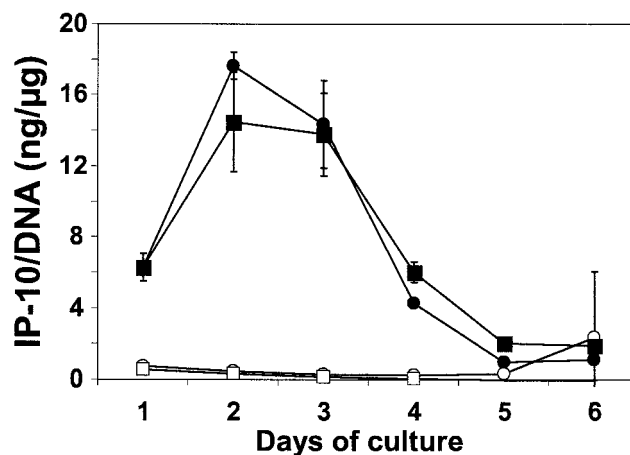


FIG. 4. Kinetics of IP-10 production of human Leydig cells exposed to mumps virus in conditioned medium. Human Leydig cells were cultured in culture medium alone (○), in the presence of 25  $\mu\text{g}$  of ribavirin per ml (□), in the presence of  $10^4$  PFU of mumps virus per ml (●), and in the presence of  $10^4$  PFU of mumps virus per ml plus 25  $\mu\text{g}$  of ribavirin per ml (■). The IP-10 concentration in the conditioned medium was determined by a specific ELISA every day. The data presented are means  $\pm$  standard errors of the means of results for three wells, each of which was assayed in duplicate, and are representative of three separate experiments.

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