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Immunology

Inflammatory mediators increase surface expression of integrin ligands, adhesion to lymphocytes, and secretion of interleukin 6 in mouse Sertoli cells

(cytokines/testis/blood–tubular barrier/intercellular adhesion molecule ICAM-1/vascular cell adhesion molecule VCAM-1)

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ABSTRACT **The expression of the cell adhesion molecules ICAM-1, ICAM-2, and VCAM-1 and the secretion of the cytokine interleukin 6 have been measured in mouse Sertoli cells cultured *in vitro*. Cytometric analysis revealed that, in basal conditions, low levels of ICAM-1 and VCAM-1 were present on the surface of the cells, whereas treatment with interleukin 1, tumor necrosis factor α, lipopolysaccharide, or interferon γ induced, with different kinetics, increases in their expression. ICAM-2 was not detectable in basal conditions, nor was it inducible. Electron microscopic analysis and binding experiments using ^{51}Cr-labeled lymphocytes demonstrated that increased expression of ICAM-1 and VCAM-1 on the surface of Sertoli cells, induced by inflammatory mediators, determines an augmented adhesion between the two cell types. The same stimuli, with the exception of interferon γ, produced a rapid and remarkable increment of interleukin 6 production by Sertoli cells. These results suggest the presence of both direct and paracrine mechanisms of interaction between Sertoli and immune-competent cells, possibly involved in the control of immune reactions in the testis. Such mechanisms are of interest for the understanding of autoimmune pathologies of the testis and, if confirmed in humans, they could be involved in the sexual transmission of human immunodeficiency virus infection.**

The testis is an immunologically privileged site of the body (1, 2). Germ cell-linked autoantigens expressed from puberty on are physiologically tolerated *in situ*, but they elicit strong autoimmune reactions if injected elsewhere in the body (3). This phenomenon has allowed researchers to use experimental autoimmune orchitis (EAO) as a tool to study the mechanisms of similar spontaneous pathologies (4).

Sertoli cells, described as "nursing cells" by their discoverer, are both the target for the hormones regulating spermatogenesis and responsible for the maintenance of the specific microenvironment in which postmeiotic development takes place. Immune tolerance in the testis has long been explained on the basis of the fact that Sertoli cells can mechanically segregate all germ cell autoantigens by means of the so called blood-tubular barrier (5). Additional information, however, revealed that this barrier isolates most, but not all, the autoantigens. Some of them escape the barrier and are available to interstitial immune cells, which nevertheless do not react against them (6). Recently we have suggested that immune tolerance in the testis may result from the synergistic operation of two mechanisms: segregation of most of the autoantigens and local production of immunosuppressive molecules by Sertoli cells (7).

Even though the mechanisms through which immune tolerance is maintained in the testis seem to be on the way to being clarified, it is still quite obscure how their perturbation leads to autoimmune diseases in the testis.

Sertoli cells could be involved in the negative as well as in the positive regulation of the immune response. In both of these phenomena, accessory cells and lymphocytes interact by means of two basic mechanisms: paracrine messages through cytokines and specific surface interactions (8). Primary cultures of Sertoli cells maintain their differentiated phenotype, including their ability to respond to follicle-stimulating hormone (FSH) (9). Cultured Sertoli cells have been demonstrated to secrete interleukin (IL)-1 (10), transferrin, and IL-6 (11) and are active phagocytes (12), but they do not constitutively express major histocompatibility complex (MHC) class II determinants or macrophage-specific markers (13). Moreover, their production of both transferrin (14) and IL-6 is enhanced by FSH stimulation (11). Thus, Sertoli cells produce a series of cytokines and factors which act on a variety of cell types and particularly on cells of the immune system. IL-6 is one of the most potent cytokines known to activate T and B lymphocytes (15, 16). Recently, the possibility has been suggested that deregulation of IL-6 gene expression may be involved in the pathogenesis of autoimmune diseases (17–19).

In this article we report that, in cultured mouse Sertoli cells, inflammatory mediators such as IL-1α, IL-1β, tumor necrosis factor α (TNF-α), and lipopolysaccharide (LPS) strongly enhance the surface expression of intercellular adhesion molecule 1 (ICAM-1) and vascular cell adhesion molecule 1 (VCAM-1), known to be specific binding molecules for lymphocytes. Such response is accompanied by a parallel increase in the secretion of biologically active IL-6.

MATERIALS AND METHODS

Sertoli Cell Cultures. Sertoli cells were prepared from CD1 mice as previously described (13). Briefly, testes from 15-day-old animals were sequentially digested with 0.25% trypsin + DNase at 10 μg/ml and then 0.1% collagenase + DNase at 10 μg/ml (Boehringer Mannheim) for 20 min to remove interstitial tissue and peritubular cells. Tubular fragments, mainly composed of Sertoli cells, were cultured at 32°C in 95% air/5%

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Abbreviations: ICAM-1, intercellular adhesion molecule 1 (CD54); VCAM-1, vascular cell adhesion molecule 1; IL, interleukin; TNF-α, tumor necrosis factor α; LPS, lipopolysaccharide; IFN-γ, interferon γ; mAb, monoclonal antibody; MHC, major histocompatibility complex; FITC, fluorescein isothiocyanate; R-PE, R-phycoerythrin; FCS, fetal calf serum; a.u., arbitrary unit(s); U, unit(s).

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CO_2 in serum-free minimum essential medium (MEM; GIBCO/BRL). Three days later, Sertoli cell monolayers were incubated at room temperature with 20 mM Tris·HCl buffer, pH 7.4, for 2 min to remove residual germ cells (20). Sertoli cell cultures were routinely checked for possible contamination by macrophages and peritubular myoid cells by indirect immunofluorescence with anti-macrophage monoclonal antibody (mAb) (Mac-1 antigen CD11/b; Boehringer Mannheim) and by histochemical detection of alkaline phosphatase activity (21). Sertoli cell cultures from 27-day-old mice were prepared as described above with minor modifications consisting in a longer incubation time (30 min) of seminiferous tubules with trypsin and collagenase.

At the fourth day of culture, Sertoli cell monolayers were treated with murine recombinant (r) TNF-α, murine recombinant interferon γ (rIFN-γ), human rIL-1α, human rIL-1β (Boehringer Mannheim), or *Escherichia coli* serotype O111:B4 LPS (Sigma) from 4 to 48 hr. At the indicated time, samples of Sertoli cell-conditioned media were collected and frozen (−20°C) before measurement of IL-6 activity by B9 cell proliferation assay, while the cells of the same samples were analyzed for ICAM-1, ICAM-2, and VCAM-1 expression by flow cytometric analysis.

Assay for IL-6 Activity. Supernatants from mouse Sertoli cells untreated or treated with TNF-α, IL-1α and -β, IFN-γ, or LPS were assayed for IL-6 activity. IL-6 was measured by using a B9 cell hybridoma growth factor assay (22) in which Sertoli cell-conditioned media were used to supplement the IL-6-dependent B9 cell line (kindly provided by Lucien Aarden, Central Laboratory of the Netherlands Red Cross, Amsterdam).

The proliferative response of B9 cells to IL-6 is expressed relative to a standard that contains known amounts of IL-6 activity. One unit of IL-6 was defined as the reciprocal of the dilution giving 50% maximal stimulation of proliferation.

Briefly, 2×10^4 B9 cells in 100 μl of complete RPMI 1640 medium (GIBCO/BRL) supplemented with 10% fetal calf serum (FCS) were added to each well of a 96-well microtiter plate and were incubated at 37°C in the presence of serial dilutions of Sertoli cell supernatants or mouse recombinant IL-6 (Genzyme). After 72 hr, the B9 cell proliferation was evaluated by [^{3}H]thymidine incorporation (0.5 μCi per well, specific activity 6.7 Ci/mmol, NEN/DuPont; 1 μCi = 37 kBq). To verify the specificity of the B9 assay, parallel samples were treated with neutralizing mAb anti-mouse IL-6 (Genzyme) at 20 ng/ml.

This assay was previously determined to be insensitive to IL-1α and -β, TNF-α, IFN-γ, and LPS at each dose used. All data points illustrated are the average of at least three wells.

Flow Cytometry. Control and treated Sertoli cells were detached with 0.02% EDTA and washed with cold Dulbecco's phosphate-buffered saline without Mg^{2+} and Ca^{2+} (PBS) + 1% bovine serum albumin (BSA). For detection of adhesion molecules on the Sertoli cell surface the following mAbs were used: fluorescein isothiocyanate (FITC)-conjugated hamster IgG anti-mouse CD54 (ICAM-1) or FITC-conjugated rat IgG2a anti-mouse VCAM-1 (INCAM-110) or R-phycoerythrin (R-PE)-conjugated rat IgG2a anti-mouse ICAM-2 (PharMingen). Specific mAbs or the appropriate isotypic control mAbs were used at 1 μg per 10^6 cells for 30 min on ice. Cells were then washed twice with PBS + 1% BSA and analyzed with an Epics 541 (Coulter) flow cytometer. Cells were gated, using forward vs. side scatter to exclude dead cells and debris.

Fluorescence of 10^4 cells per sample was acquired in logarithmic mode for visual inspection of the distributions and in linear mode for quantitating the expression of the relevant molecules.

Preparation of Splenic Lymphocytes. Spleens from adult CD1 mice were aseptically removed and gently dissociated in culture medium. Red blood cells were eliminated by treatment with ACK-lysing buffer (GIBCO/BRL) and the cell suspension was enriched in T lymphocytes by incubation on a nylon wool column (Polyscience) for 1 hr at 37°C (23). Cytometric analysis of this population using anti-CD3 mAbs revealed that T lymphocytes accounted for about 50% of the total. Lymphocytes were then resuspended in RPMI 1640 medium supplemented with 10% FCS, 2 mM glutamine, 50 μM 2-mercaptoethanol, and gentamicin at 50 μg/ml.

Splenic Lymphocyte Adhesion Assay. Sertoli cells were plated in Lab-tek tissue culture chamber slides (Nunc) and treated with TNF-α (20 ng/ml) for 20 hr at 32°C, then TNF-α was removed before cells were used for the binding experiments.

Adhesion of lymphocytes to Sertoli cell monolayers was assessed by using ^{51}Cr-labeled lymphocytes in a ^{51}Cr retention assay modified from that described previously (24). Briefly, splenic lymphocytes were labeled with ^{51}Cr (sodium chromate NEN/DuPont) for 1 hr at 37°C in 200 μl of FCS. Labeled cells, washed twice and resuspended at 8×10^6 cells per ml in RPMI 1640 complete medium containing 10% FCS, were treated with phorbol 12-myristate 13-acetate (PMA) at 50 ng/ml for 30 min at 37°C to enhance avidity of leukocyte function-associated antigen 1 (LFA-1) (25). Lymphocytes were then washed and incubated (8×10^5 cells per well) at 37°C for 75 min with Sertoli cell monolayers. Unbound lymphocytes were removed by dipping the culture slides twice in Hanks' buffer, bound lymphocytes were lysed with 5% Triton X-100, and released radioactivity was measured by using a γ counter. All assays were with four replicates, and the number of adhering lymphocytes was calculated on the basis of the specific activity of the lymphocytes added.

For binding inhibition experiments, Sertoli cells in tissue culture chamber slides were preincubated with anti-ICAM-1 and anti-VCAM-1 mAbs at various concentrations for 2 hr before addition of ^{51}Cr-labeled lymphocytes. Isotype-matched IgG2a or hamster irrelevant mAbs were used as a control.

Scanning Electron Microscopy. For scanning electron microscopic observations, Sertoli cells were plated onto glass coverslips, treated with TNF-α at 20 ng/ml for 20 hr, and incubated with splenic lymphocytes at 37°C for 75 min as reported above. After removal of nonadhering lymphocytes, Sertoli cells were fixed in 2.5% glutaraldehyde in 0.1 M sodium cacodylate buffer (pH 7.2) for 2 hr. After a thorough wash in cacodylate buffer, the samples were postfixed in 1% aqueous OsO_4 for 40 min, dehydrated in absolute ethanol, and dried by the critical-point drying method. Specimens were examined under a Hitachi S570 scanning electron microscope.

RESULTS

Effects of Inflammatory Mediators on the Expression of Adhesion Molecules on Sertoli Cells. The surface expression of ICAM-1, ICAM-2, and VCAM-1 has been measured by flow cytometric analysis on *in vitro* cultured Sertoli cells from 15-day-old mice.

Prepuberal Sertoli cells did not exhibit the presence of ICAM-2 on their surface, whereas small but significant

Table 1. Basal expression of adhesion molecules in mouse Sertoli cells

Antibody	Fluorescence, a.u.	P*
FITC hamster IgG isotype standard mAb	25 ± 2	
FITC hamster anti-mouse ICAM-1	34 ± 1	<0.01
R-PE rat IgG2a, κ isotype standard mAb	14 ± 1	
R-PE rat IgG2a anti-mouse ICAM-2	14 ± 1	NS
FITC rat IgG2a, κ isotype standard mAb	22 ± 1	
FITC rat IgG2a anti-mouse VCAM-1	26 ± 2	<0.05

Results are presented as mean ± range; a.u., arbitrary units.
*Differences between test mAb and isotypic control mAb have been determined by Lord's t test (26); NS, not significant.

amounts of ICAM-1 and VCAM-1 were detected (Table 1). When the cells were treated for 24 hr with a panel of cytokines (TNF-α, IL-1α, IL-1β, IFN-γ, or LPS), known to mediate inflammatory response, they responded, to various extents, by increasing the expression of both ICAM-1 and VCAM-1 (Fig. 1) but not that of ICAM-2 (not shown). From flow cytometric profiles of both treated and untreated samples, it appears that surface molecules are homogeneously expressed in the cell population. The mean fluorescences, acquired in linear mode on triplicate samples in a representative experiment, are reported in Table 2. The expression of both ICAM-1 and VCAM-1 is significantly increased, even if to a variable extent, by all the treatments tested. The increase of expression was variable in different experiments, probably due to the complex procedure of cell preparation. For instance, the net (i.e., after subtraction of background) expression of ICAM-1 after the treatment with TNF-α at 20 ng/ml ranged from 19 to 31 times the control value. However, the reproducibility within the same experiment was always good, the relative error in triplicate samples seldom exceeding 5%.

Since TNF-α was the most effective factor tested for the induction of adhesion molecules, we have performed dose–response and time-course experiments with this cytokine. TNF-α induced, within 24 hr, a dose–response increment in the surface expression of the lymphocyte adhesion molecules ICAM-1 and VCAM-1 on Sertoli cell cultures. Maximal stimulation of both ICAM-1 and VCAM-1 expression with TNF-α was obtained at a concentration of about 20 ng/ml (Fig. 2*A*).

Semiquantitative analysis by immunofluorescence showed that not only prepuberal Sertoli cells but also Sertoli cell cultures from young adult mice exhibit a similar response to TNF-α (not shown). In Fig. 2*B* it can be seen that Sertoli cells respond to the stimulus as early as after 4 hr of treatment, reaching the maximal concentration of ICAM-1 and VCAM-1 on their surface between 16 and 24 hr of treatment.

Role of ICAM-1 and VCAM-1 in Lymphocyte Adhesion to Cytokine-Stimulated Sertoli Cells. Binding experiments have been employed to detect the actual ability of lymphocytes to specifically adhere to Sertoli cells untreated or treated with TNF-α. Fig. 3 shows that stimulation of Sertoli cells with TNF-α increased their ability to bind activated lymphocytes.

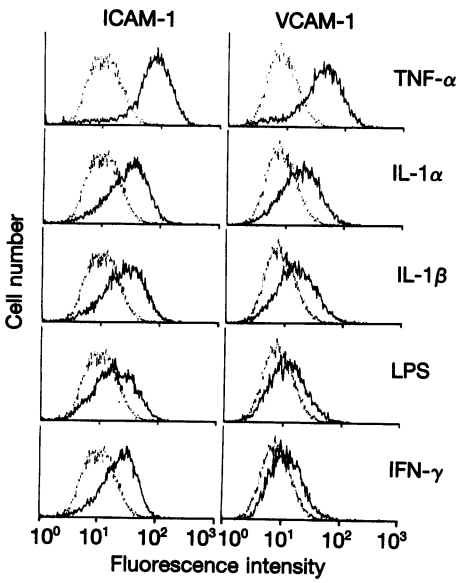

FIG. 1. Flow cytometric analysis of the expression of ICAM-1 and VCAM-1 in mouse Sertoli cells. Cells were stimulated for 24 hr with TNF-α (20 ng/ml), IL-1α [10 units (U)/ml], IL-1β (10 U/ml), LPS (500 ng/ml), or IFN-γ (500 U/ml). Broken lines, untreated cells; continuous lines, treated cells.

Table 2. Effect of different treatments on the expression of adhesion molecules in mouse Sertoli cells

Treatment	Fluorescence, a.u. ICAM-1	VCAM-1
Isotypic antibody	25 ± 1	24 ± 1
Control	36 ± 1	28 ± 1
TNF-α (20 ng/ml)	245 ± 7	131 ± 4
IL-1α (10 U/ml)	93 ± 5	62 ± 3
IL-1β (10 U/ml)	86 ± 1	57 ± 2
LPS (500 ng/ml)	61 ± 2	41 ± 2
IFN-γ (500 ng/ml)	72 ± 2	40 ± 1

Expression of different molecules is evaluated by flow cytometric analysis as the fluorescence intensity (linear scale; mean ± range) of specific antibodies. Fluorescence of isotypic antibody is not affected by the various treatments. Control, untreated Sertoli cells. All differences in fluorescence relative to the controls are significant at 1% probability level by Lord's *t* test (26).

About 50% of the lymphocytes added were T lymphocytes (see *Materials and Methods*). Immunofluorescence search for CD3-positive cells among those adhering to Sertoli cell monolayers confirmed this ratio. To obtain a quantitative evaluation of this effect, we performed similar experiments, using ^{51}Cr-labeled lymphocytes, and found that treatment with TNF-α doubled the binding of lymphocytes to Sertoli cell monolayers. Such response is related to the augmented expression of these adhesion molecules, since anti-ICAM-1 and anti-VCAM-1 neutralizing antibodies strongly inhibited the binding of lymphocytes to stimulated Sertoli cells (Fig. 4).

Production of Biologically Active IL-6 by Sertoli Cells and Its Modulation by Cytokines. We have also measured the production of biologically active IL-6 by Sertoli cells. TNF-α increased the basal production of IL-6 in a dose-dependent manner (Fig. 5*A*). A similar effect was obtained when IL-1α, IL-1β, or LPS was used (not shown). Treatment with IFN-γ induced a significant decrease of the basal secretion of IL-6 (Fig. 5*B*). IL-6 activity was evaluated as a function of the growth of the IL-6-dependent cell line B9, and the specificity was assessed with anti-IL-6 neutralizing antibodies. Time-course experiments demonstrated that, during the first 24 hr of treatment with TNF-α at 20 ng/ml, increased production of IL-6 parallels the augmented expression of ICAM-1 and VCAM-1 (Figs. 6 and 2*B*).

DISCUSSION

We demonstrated that mouse Sertoli cells cultured *in vitro* respond to treatments with inflammatory mediators, such as TNF-α, IL-1α, IL-1β, and LPS, by increasing the expression of ICAM-1 and of VCAM-1 on their surface. This pattern of cytokine-upregulated expression is in agreement with previous

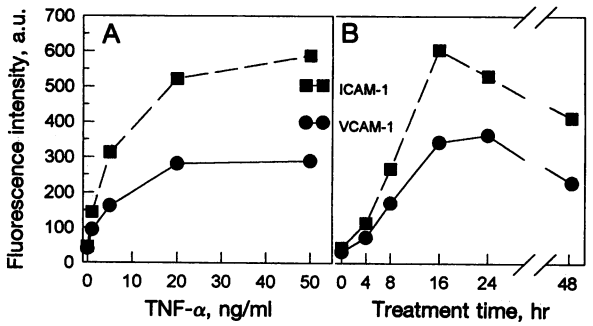

FIG. 2. (*A*) Dose–response curves for the expression of adhesion molecules ICAM-1 (■) and VCAM-1 (●) on Sertoli cells after 24 hr of treatment with TNF-α. (*B*) Kinetics of TNF-α (20 ng/ml) -induced expression of ICAM-1 (■) and VCAM-1 (●) on Sertoli cells.

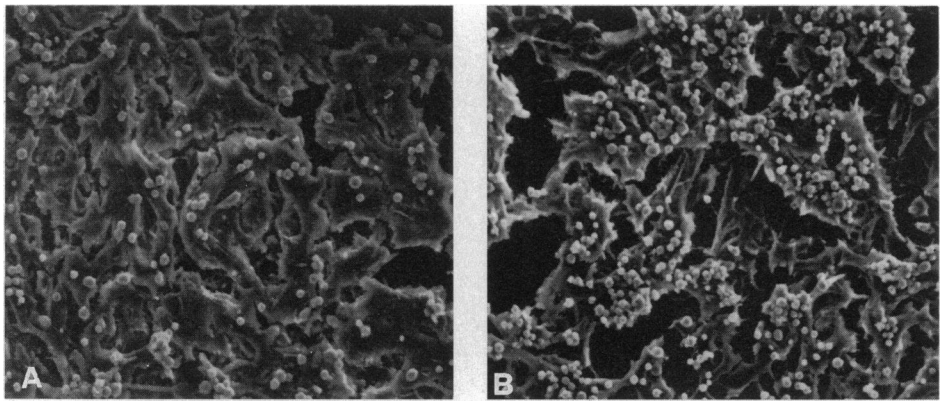

FIG. 3. Scanning electron micrographs of splenic lymphocytes adhering to Sertoli cell monolayers untreated (*A*) or stimulated for 20 hr with TNF-α at 20 ng/ml (*B*). (×470.)

studies using other cell types (27). The extent of the response to TNF-α in the Sertoli cell cultures is in the same range of that obtained with TNF-α or with other factors in the most responsive systems, including endothelial cells (28, 29). Such responses are associated with an augmented specific adhesion of lymphocytes to the surface of Sertoli cells and to a contemporary enhanced secretion of IL-6. The adhesion mechanisms involve both ICAM-1 and VCAM-1, since neutralizing mAbs to one or the other adhesion molecule strongly inhibited the increase of the binding.

Our data show that IFN-γ enhances the expression of ICAM-1 and VCAM-1 but, differently from the other factors tested, inhibits rather than stimulates the secretion of biologically active IL-6 by Sertoli cells. A reduced production of IL-6 after IFN-γ treatment has been observed in rat granulosa cells also (30). These results suggest that the regulation of the expression of adhesion molecules and of the production of IL-6 could follow, in our system, separate pathways.

It has been proposed that IL-6 secretion by rat Sertoli cells might be relevant in the paracrine regulation of spermatogenesis (11). Gene targeting has been recently used to produce mice deficient in ICAM-1 (31) or IL-6 (32). These animals exhibit, respectively, prominent abnormalities in inflammatory responses and impaired immune and acute-phase responses. These data confirm, therefore, the key role of adhesion molecules and IL-6 in both lymphocyte inflammatory response and lymphocyte activation. However, mice deficient for IL-6 are fertile, indicating that IL-6 does not play any important role in the regulation of testicular functions.

Adhesion and migration of leukocytes through the capillary wall, during the inflammatory process, implies a series of different steps. "Rolling" of leukocytes on the wall, adhesion, and, finally, migration to the connective tissue require the progressive expression of various adhesion molecules on the surface of endothelial cells (33). ICAM-1 and VCAM-1 are typically expressed by endothelial cells, but they are occasionally inducible by inflammatory stimuli in a set of nonvascular cells (34, 35), more restricted for VCAM-1 (36, 37). These molecules are considered responsible for adhesion to endothelial cells and migration of leukocytes from the blood stream to inflamed tissues (33).

An increasing body of evidence suggests that nonendothelial cells of the various tissues can be involved in the local accumulation of leukocytes and in the regulation of the immune response. It has been recently shown that inflammatory stimuli can induce, for instance, human myoblasts or keratinocytes to trigger or enhance immune reactions through the expression of specific surface molecules and the secretion of cytokines which activate T lymphocytes (38, 39).

The Sertoli cell is involved in the hormonal regulation of spermatogenesis, but it is also responsible for a series of other functions, including the formation and the maintenance of the blood–tubular barrier, the control of the tubular microenvironment, the regulation of spermiation, and the removal of abnormal germ cells (9). In addition to that, Sertoli cells are implicated in the immune tolerance of testicular autoantigens, not only by segregating them within the blood–tubular barrier but also by secreting an immunosuppressive factor(s) (7). Previous studies, attempting to characterize Sertoli cells as antigen-presenting cells, have shown the presence on their surface of class I MHC antigens, but failed to demonstrate the expression of class II determinants specific for accessory cells such as dendritic cells (13).

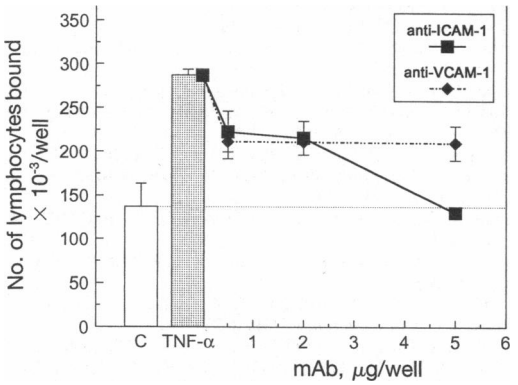

FIG. 4. Effect of anti-ICAM-1 (■) or anti-VCAM-1 (◆) mAb on the binding of splenic lymphocytes to Sertoli cell monolayers treated with TNF-α (20 ng/ml). Isotype-matched IgG2a or hamster IgG irrelevant mAbs were used as control and were ineffective. All points were assayed in groups of four replicates.

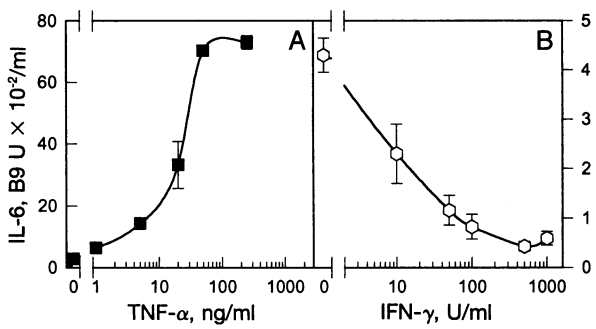

FIG. 5. Production of IL-6 by Sertoli cells stimulated for 24 hr with increasing doses of TNF-α (*A*) or IFN-γ (*B*). Conditioned media of cultures were assayed for IL-6 activity by using the proliferative response of B9 hybridoma cells. Each point represents the mean of quadruplicate samples of at least three experiments.

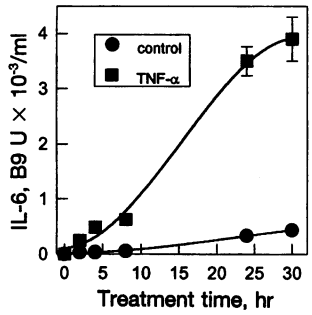

FIG. 6. Time course of IL-6 production by Sertoli cells stimulated with TNF-α (20 ng/ml). Values are the means of triplicate samples and are representative of at least three experiments.

One of the most intriguing questions regarding the pathogenesis of autoimmune disorders of the testis (40) refers to the mechanisms by which lymphocytes cross the blood–testis barrier and reach immunogenic germ cells, causing a peritubular and intratubular leukocyte infiltration (41). It has been recently demonstrated that autoimmune orchitis can be induced by transferring testis antigen-specific T-cell clones to normal mice (42). Interestingly enough, disease transfer was abolished when recipients were injected with neutralizing antibody to TNF-α, suggesting that TNF-α can be considered a cytokine responsible, or at least important, in the pathogenesis of this autoimmune disease. Other authors suggested that TNF-α release at the various sites of the body could cause opening of tight junctions and a breakdown in the barrier function of an epithelial cell sheet (43).

Our data bring new insights on the possible pathogenic mechanisms of autoimmune disorders of the testis. We demonstrated that Sertoli cells respond to inflammatory mediators, and maximally to TNF-α, by increasing their ability to adhere to both T and B lymphocytes and by secreting biologically active IL-6. The response of Sertoli cells to inflammatory factors could contribute to the activation of lymphocytes and to their migration throughout the blood–tubular barrier to reach autoantigen-bearing germ cells within the seminiferous epithelium. The knowledge of these properties of Sertoli cells could be relevant to the comprehension of the pathogenesis of those immunological disorders of the testis resulting in an impaired fertility (44).

Nuovo *et al.* (45) recently reported that the presence of human immunodeficiency virus (HIV) in the central nervous system is associated with an upregulation of TNF-α transcription. We demonstrated that mouse Sertoli cells respond to TNF-α by increasing their specific adhesion to lymphocytes. These functional responses of mouse Sertoli cells, if proved to exist also in the human testis, could help in understanding the mechanisms underlying the sexual transmission of HIV infection. Increased levels of TNF-α, associated with HIV infection, could augment the expression of leukocyte adhesion molecules on the surface of Sertoli cells and loosen the intercellular tight junctions. Virus-infected lymphocytes could therefore migrate through the seminiferous epithelium toward the tubular lumen and eventually to the seminal fluid.

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