Immunopathology of Primary Hypophysitis
Implications for Pathogenesis

A. Gutenberg, MD, R. Buslei, MD,† R. Fahlbusch, MD,‡ M. Buchfelder, MD,§ and W. Brück, MD**

Abstract: The etiology of primary hypophysitis is still not fully elucidated. Histologically, primary hypophysitis includes three different main subtypes: lymphocytic (LYH), granulomatous (GRH), and xanthomatous (XH) hypophysitis. Clinical and laboratory findings suggest an autoimmune basis in primary hypophysitis. Controversy still exists about the composition of the inflammatory infiltrate and the relevant immunopathogenic effector mechanisms. Therefore, 21 cases of primary hypophysitis of different subtypes were analyzed with respect to the expression of lymphocyte and macrophage antigens as well as MHC class I and II molecules of the inflammatory infiltrate and the resident pituitary acinar cells. Lymphocyte infiltration in LYH (n = 15), but also in GRH (n = 4) and XH (n = 2), mainly consisted of T cells, while B cells were rare. Independent from the histopathologic subtype, T cell subsets showed equal ratios of CD4+ to CD8+ T cells. Highest numbers of activated CD8+ T cells were observed in LYH presenting during pregnancy, surrounding or even infiltrating preserved pituitary acinar cells. Moreover, an increased rate of activated CD8+ T cells correlated with a shorter duration of clinical symptoms. In LYH, aberrant expression of MHC class II antigens as well as overexpression of MHC class I molecules on pituitary cells were observed. Independent of the histologic subtype, macrophages mostly expressed markers of chronic activation and showed MHC class II positivity. LYH, GRH, and XH, although heterogeneous in their histologic appearance and in age distribution, exhibit a similar if not identical immunohistologic profile. It is highly likely that direct T cell-mediated cytotoxicity through CD8+ T cells, with the initial help of CD4+ T cells, is pivotal in the pathogenesis of primary hypophysitis, implicating a target autoantigen expressed by pituitary cells.

Key Words: hypophysitis, T-cells, macrophages

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Primary hypophysitis is an important differential diagnosis in space-occupying lesions of the sella region. Histologically, primary hypophysitis includes three different types: lymphocytic hypophysitis (LYH), granulomatous hypophysitis (GRH), and xanthomatous hypophysitis (XH).[8] Some authors classify xanthogranulomatous hypophysitis (XGH) and necrotizing hypophysitis (NH) as further autonomous entities.[1,17,34] Pathogenesis of primary hypophysitis is not fully understood.[2,3,20] It is unclear whether these histologic subtypes are truly different entities or only different expressions of the same disease. LYH is assumed to have an autoimmune basis most often occurring in young women, typically late in pregnancy or during the first 6 months of the postpartum period.[2] However, it also occurs in nonpregnant women or even in men at a female-to-male ratio of 8:1.[20,21,26] An autoimmune pathogenesis is supported by several clinical and laboratory findings. One third of patients suffer from additional autoimmune diseases (eg, Hashimoto thyroiditis, adrenalitis, Sjögren's syndrome, autoimmune gastritis, and hepatitis).[35] Anti-pituitary hormone antibodies in sera from patients with LYH, as well as specific anti-pituitary antibodies to a 22 kDa and a 49 kDa antigen, could also be detected, identifying them as human growth hormone and alpha- and gamma-enolase, respectively.[2,9,27,28,32,33] In contrast to LYH, no sex ratio is reported for GRH and XH. Neither occurs during pregnancy, and there is no evidence for associated autoimmune diseases.[3,7,8,12,15,34]

Histopathologic examination of inflammatory infiltrates, mainly of LYH and GRH, has been carried out in many studies.[13,14,34] Controversy still exists about the composition of the inflammatory infiltrate. Infiltrating lymphocytes in LYH and GRH have mainly been reported as T cells, with a predominance of CD4+ over CD8+ positive T cells.[3,13] In other studies, lymphocytes consisted of an equal ratio of B and T cells.[34] Macrophages also contribute significantly to the inflammatory infiltrate in primary hypophysitis and presumably lead to destruction of the pituitary gland. Macrophages can be characterized by activation-associated antigens expressed during the early or late phase of inflammation. The expression of the myeloid-related protein MRP14 and the heterodimer of MRP8/14, the 27E10 epitope,[4] is associated with acute inflammatory activated macrophages, expressing proinflammatory cytokines such as tumor necrosis factor-α and oxygen radicals as cytotoxic factors.[5] In contrast, the MRP8 and 25F9 antigens are expressed by macrophages during the late phase of chronic inflammation,[30,37] representing resident activated macrophages. So far, there are no data available on their differentiation or activation stage in primary hypophysitis.

MHC class II molecules are classically expressed by professional antigen-presenting cells, such as dendritic cells, macrophages, and B cells. Their main function is to present antigen to CD4+ T cells[6] and thus trigger a T cellular immune response. Increased endothelial expression of MHC class II molecules has been demonstrated in a number of idiopathic autoimmune disorders, such as juvenile diabetes mellitus, rheumatoid

From the Departments of *Neuropathology and §Neurosurgery, Georg August University, Göttingen, Germany; and Departments of †Neuropathology and ‡Neurosurgery, Friedrich Alexander University, Erlangen, Germany.
Reprints: Angelika Gutenberg, MD, Department of Neuropathology, Georg August University, Robert-Koch-Str. 40, D-37075 Göttingen, Germany (e-mail: agutenberg@med.uni-goettingen.de).

arthritis, and systemic lupus erythematodes.[36] Aberrant expression has not yet been shown for primary hypophysitis.[25]

To obtain further information on the pathogenesis of primary hypophysitis, 21 cases of different subtypes of primary hypophysitis were analyzed with respect to lymphocyte and macrophage antigens. Additionally, expression of MHC class I and II molecules of the inflammatory and pituitary acinar cells were investigated.

MATERIALS AND METHODS

Twenty-one formalin-fixed, paraffin-embedded hypophysitis biopsy specimens from 20 patients were investigated. Tissue samples were obtained during transsphenoidal surgery from 1992 to 2004 at the Departments of Neurosurgery of the University Hospitals of Erlangen and Göttingen (Germany). Standard hematoxylin and eosin stain and periodic acid-Schiff reaction were used for histologic examination. To exclude

TABLE 1. Diagnosis and Main Clinical Data of 20 Patients With Primary Hypophysitis

Patient No.	Diagnosis	Age (yr)	Sex	Presenting Symptoms	Autoimmune Disease
1	Lymphocytic	27	F	3 months of diabetes insipidus, frontal headaches	Wegener granulomatosis, asthma bronchiale
2	Lymphocytic	27	F	2 months of headache at the end of pregnancy, visual field defect	—
3	Lymphocytic	28	F	1 week of progredient visual deterioration in the last month of pregnancy	—
4	Lymphocytic	32	M	5 months of diabetes insipidus, impotence, depressions	—
5	Lymphocytic	33	F	2 months of diabetes insipidus, headache, and vomiting in the last 2 months of pregnancy, visual deterioration, and bitemporal hemianopsia	—
6	Lymphocytic	33	M	9 years before operation due to inflammatory granuloma of the pituitary, 5 years of chiasma syndrome, 6 months of visual deterioration	—
7	Lymphocytic	34	M	3 weeks of left frontal headache	Psoriasis
7a	Lymphocytic recurrency	37	M	Few days of left N. VI palsy	Psoriasis
8	Lymphocytic	37	M	Coma diabeticum, incidental diagnosis of pituitary mass	Juvenile diabetes mellitus
9	Lymphocytic	42	F	12 months of headache, 6 months of galactorrhea and amenorrhea	—
10	Lymphocytic	42	M	6 years of known pituitary insufficiency, 12 months of headache, impotence, and depressions	—
11	Lymphocytic	43	F	40 months of diabetes insipidus, amenorrhea, and headache	—
12*	Lymphocytic	52	F	5 months of headache and right eye pain, 3 months of fatigue	—
13	Lymphocytic	61	M	2 months of headache, loss of libido and impotence, 2 weeks of right N. III palsy	—
14	Lymphocytic infundibuloneurohypophysitis	9	F	2 months diabetes insipidus	—
15	Idiopathic granulomatous	35	M	2 years of impotence, loss of libido, 15 months of fever, diarrhea, joint pain	—
16	Idiopathic granulomatous	41	M	Few days right N. VI palsy, headache, bitemporal hemianopsia	—
17*	Idiopathic granulomatous	48	F	8 months of diabetes insipidus, headache, and vomiting, progredient visual deterioration	—
18	Idiopathic granulomatous	76	M	1 month of headache and vomiting, fatigue	—
19†	Xanthomatous	30	F	Oligomenorrhoea since age 14, 6 months of chills in the right extremities, right trigeminal hypaesthesia, left body hypaesthesia, tinnitus, retroorbital pain	—
20	Xanthomatous	39	M	9 months of diabetes insipidus, impotence, and loss of libido, headache	—

*Case nos. 12 and 17 published by Honegger et al.[20]
†Case no. 19 published by Deodhare et al.[12]

tuberculosis and fungus infection in GRH cases, Ziehl-Neelsen and Grocott methamine silver stainings were performed.

Immunohistochemistry was applied to characterize the cellular composition of pituitary inflammation. Immunostainings were made for CD3+, recognizing T cells (1:200 dilution, monoclonal; Serotec, Oxford, UK), CD8+, detecting cytotoxic and suppressor T cells (1:50 dilution, monoclonal, Dako, Hamburg, Germany), granzyme B, labeling activated cytotoxic T cells (1:50 dilution, monoclonal, Dako), CD20+, recognizing B cells (1:100 dilution, monoclonal, Dako), CD79a, detecting mature B and plasma cells (1:50 dilution, monoclonal, Dako), Ki-M1P, recognizing all macrophages (1:200 dilution, a generous gift of Prof. H. J. Radzun, Göttingen, Germany), MRP14 (1:1000 dilution, monoclonal, BMA, Augst, Switzerland), and 27E10 (1:50 dilution, monoclonal, Dianova, Hamburg, Germany), recognizing early activated macrophages, MRP8 (1:1000 dilution, monoclonal, BMA) and 25F9 (1:50 dilution, monoclonal, Dianova), recognizing inflammatory macrophages late during inflammation, C9neo, labeling activated lytic or terminal complement complex (1:50 dilution, monoclonal; 1:1000 dilution, polyclonal, Department of Biochemistry Cardiff, UK), HC-10 (1:500 dilution, monoclonal, a generous gift from Prof. H. Pluegh, Harvard Medical School, Boston, MA), recognizing HLA-A, -B, C molecules, and CR3/43 (1:100 dilution, monoclonal, Dako) recognizing HLA-DP, -DQ and -DR molecules; 3-μm-thick formalin-fixed, paraffin-embedded sections were performed using the labeled streptavidin-biotin method (Dako), after deparaffinization, rehydration, and blockade of endogenous peroxidase activity. Sections to be stained for CD3+, CD8+, CD79a, granzyme B, Ki-M1P, MRP14, 7MRP8, 7HC-10, CR3/43, and C9neo (monoclonal) underwent microwave antigen retrieval in a 0.1 mM citrate buffer for 15 minutes. Tissue sections for 27E10, 25F9, and C9neo (polyclonal) were pretreated with 10 μg/mL proteinase K (Dako) in phosphate-buffered saline for 10 minutes at 37°C for antigen retrieval. Tissue sections to be stained for CD20+ were not pretreated. All sections were incubated at 4°C overnight with the primary antibody, and for 1 hour each at room temperature with biotinylated secondary antibody and peroxidase-labeled streptavidin. 3′-3′-diaminobenzidine (Sigma) served as the chromogen. Slides were then counterstained with hematoxylin. Five pituitaries removed at autopsy within 24 hours after death served as controls. All 5 patients died of nonendocrine or nonseptic causes.

A histomorphometric study was undertaken in a total of at least 10 randomly selected 400-fold high power fields. Statistical analyses were performed using the Mann-Whitney *U* test to correlate clinical and histopathologic data and the Kruskal-Wallis test to compare histologic features between different subtypes of hypophysitis. Two-tailed *P* values of <0.05 were considered statistically significant.

RESULTS

Clinical Presentation

A total of 21 biopsy specimens from 20 patients with primary hypophysitis were examined. Main clinical features of patients represented in this study are summarized in Table 1.

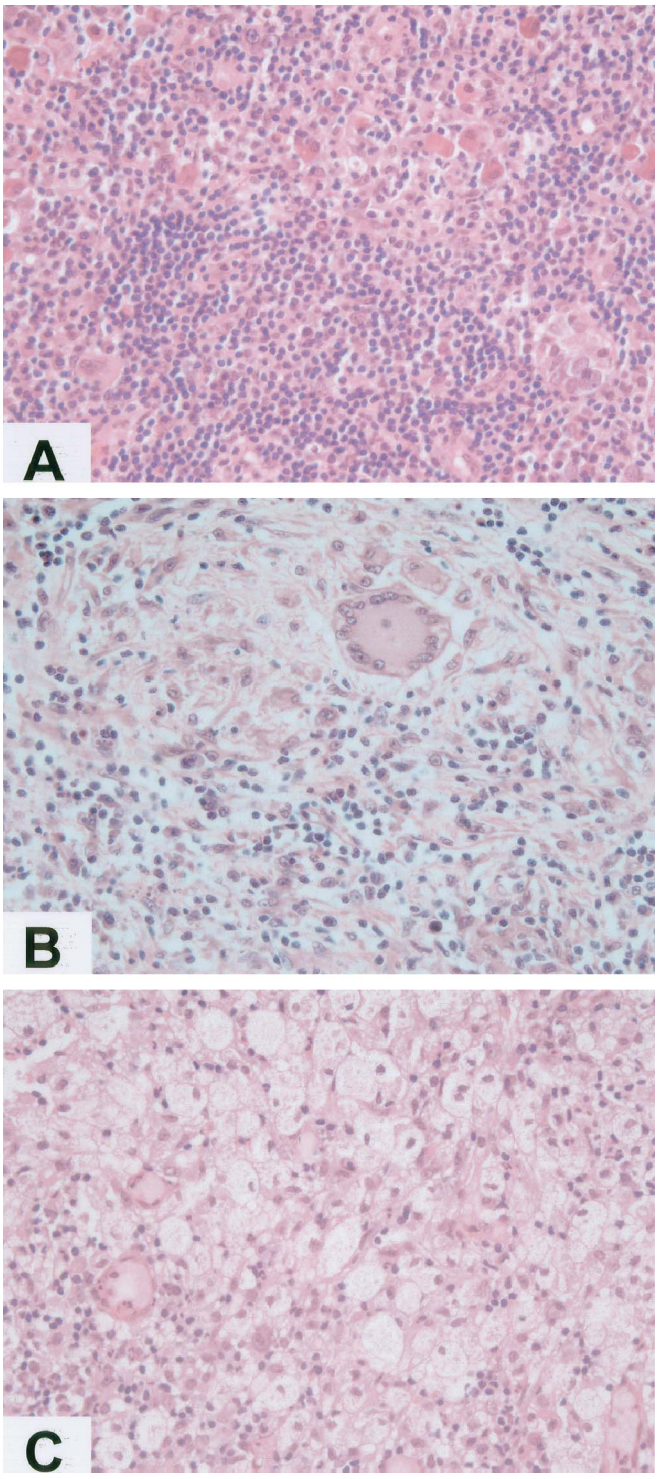

FIGURE 1. Histologic subtypes of primary hypophysitis. A, Lymphocytic hypophysitis. Note massive lymphocytic infiltration of pituitary with scattered islands of preserved pituitary cells. B, Idiopathic granulomatous hypophysitis. Characteristic multinucleated giant cells and granuloma surrounded by fibrosis, sparse infiltration of plasma cells. C, Xanthomatous hypophysitis. Predominance of foamy macrophages, a few lymphocytes, and single plasma cells (hematoxylin and eosin, original magnification ×40).

Ten patients were female and 10 were male, and no gender predominance was observed for any histologic type of primary hypophysitis. Patient age ranged from 9 to 76 years (mean, 38.4 years; median, 37.0 years). Male patients were slightly older than female patients (mean age, 33.9 vs. 43.0 years). Mean age of patients with LYH was 35.7 years, whereas patients with GRH were older (mean, 50.0 years), and patients with XH were slightly younger (mean age, 34.5 years). The duration of symptoms before pituitary biopsy ranged from a few days to more than 9 years. In 9 patients (case nos. 2, 3, 5, 7, 8, 13, 14, 16, and 18), biopsy samples were taken within 8 weeks after beginning of symptoms. Headache was the most frequent complaint (14 of 20 patients), followed by visual field impairment (6 of 20 patients). Endocrinologic disorders were most often diabetes insipidus and impairment of the gonadal axis. Neurologically, a third nerve palsy was found in case no. 13; case nos. 7a and 14 presented with a sixth nerve palsy. In case no. 19, a hemihypaesthesia of the left body side was observed. In 3 of 10 female patients (case nos. 2, 3, and 5), hypophysitis was associated with pregnancy, being manifest in the last trimester of pregnancy. Three patients (case nos. 1, 7, and 8) additionally had autoimmune disorders. Interestingly, only LYH was associated with pregnancy and autoimmune disorders. Patient no. 6 was known to have been operated on for "inflammatory granuloma" of the pituitary gland 9 years earlier (no sample available); and in patient no. 7, LYH recurred as lymphocytic again 23 months after the first operation (case no. 7a). In patients with GRH, systemic granulomatous disorders such as tuberculosis, sarcoidosis or Wegener's granulomatosis were excluded clinically.

Histologic Findings

All cases showed classic light microscopic features of LYH (n = 15), GRH (n = 4), or XH (n = 2) (Fig. 1, Table 2). In all cases of LYH, pituitary tissue was diffusely infiltrated by high numbers of inflammatory cells consisting mostly of lymphocytes, plasma cells, and some macrophages. In 5 of 15 cases of LYH, lymphocytes were arranged in aggregates, none with germinal centers. Case nos. 5 and 8 additionally showed an acute inflammatory infiltrate with intermixed neutrophil granulocytes. In all but 5 cases, scattered islands of preserved pituitary cells were seen, whereas the remaining pituitary tissue showed a variable amount of reactive interstitial fibrosis. Normal anterior pituitary was seen in case no. 13, showing inflammation solely in the neurohypophysis and infundibulum. Therefore, case no. 13 was diagnosed as lymphocytic infundibuloneurohypophysitis. In case no. 11, a few scattered xanthomatous macrophages were also seen, but apart from this case, no foreign body giant cells, xanthomatous macrophages, or necrosis were seen in cases of LYH.

In all cases classified as GRH, there were many multinucleated giant cells, while varying numbers of lymphocytes and plasma cells were present in the surrounding inflamed tissue. Lymphoid aggregates without germinal centers were also seen in 3 of 4 GRH cases. All cases showed a lobular configuration of fibrotic areas, leaving variable numbers of

TABLE 2. Main Histomorphologic Alterations in Different Subtypes of Primary Hypophysitis

Patient No.	Diagnosis	Amount of Plasma Cells	Fibrosis	Multinucleated Giant Cells	Xanthomatous Macrophages	Necrosis
1	Lymphocytic	+ +	+	−	−	−
2	Lymphocytic	+	+ + +	−	−	−
3	Lymphocytic	+ +	−	−	−	−
4	Lymphocytic	(+)	−	−	−	−
5	Lymphocytic	+ +	−	−	−	−
6	Lymphocytic	+	+ + +	−	−	−
7	Lymphocytic	−	+	−	−	−
7a	Lymphocytic recurrency	+	+ +	−	−	−
8	Lymphocytic	+	+	−	−	−
9	Lymphocytic	+ +	+ + +	−	−	−
10	Lymphocytic	+ +	−	−	−	−
11	Lymphocytic	(+)	+ +	−	+	−
12*	Lymphocytic	+ + +	+ + +	−	−	−
13	Lymphocytic	(+)	+ + +	−	−	−
14	Lymphocytic infundibuloneurohypophysitis	−	−	−	−	−
15	Granulomatous	+	+ +	+	−	−
16	Granulomatous	+ + +	+ +	+ +	−	−
17*	Granulomatous	+	+ + +	+ + +	−	−
18	Granulomatous	(+)	+ +	+ +	+	+ +
19†	Xanthomatous	(+)	+	−	+ + +	−
20	Xanthomatous	−	−	−	+ + +	−

−, none; (+), single; +, few; ++, moderate; +++, many.
*Case nos. 12 and 17 published by Honegger et al.[20]
†Case no. 19 published by Deodhare et al.[12]

pituitary cells within. Case no. 18 additionally presented with xanthomatous macrophages and foci of necrosis. In none of the GRH cases were specific stains for acid-fast bacilli and fungi found.

Case nos. 19 and 20 were classified as XH, consisting predominantly of foamy macrophages mixed with sparse lymphocytes and single plasma cells. Scattered islands of residual anterior pituitary acinar cells were observed in case no. 20.

Immunohistochemical Findings

To obtain baseline information on the lymphocytic components of inflammatory infiltrates, CD3+ for the total numbers of T cells, CD8+ for cytotoxic T cells, CD20+ for B cells, and CD79a for B and plasma cells were analyzed. The absolute numbers of lymphocytes/mm^2 were highest in LYH, lower in GRH, and lowest in XH (Figs. 2, 3). Moreover, in all cases, the number of CD3+ T cells correlated significantly with fibrosis, showing decreasing cellularity with higher amount of fibrosis (P = 0.05). LYH, GRH, and XH consisted of mainly CD3+ T cells, while CD20+ B cells were rare. No lymphocytes were seen in pituitary controls.

In all but 3 cases, the CD8+/CD3+ T cell ratios ranged between 0.2 and 0.7, with a mean value of 0.5. In 3 cases (case nos. 10, 12, and 13, all LYH), CD8+ T cells clearly predominated

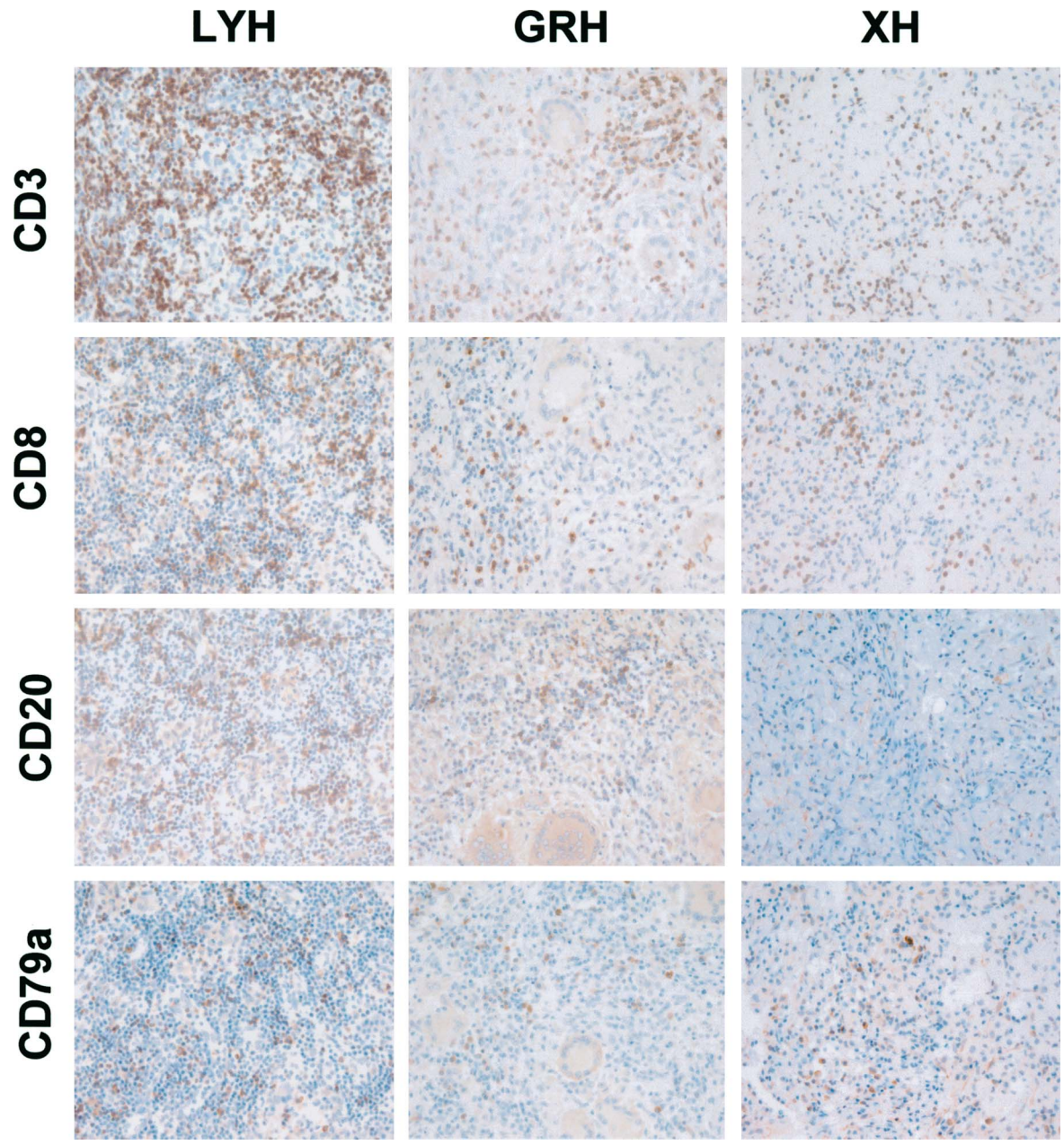

FIGURE 2. Immunohistochemical stainings of lymphocyte antigens. Highest density of lymphocytes in LYH, less in GRH, and the least infiltration by lymphocytes in XH (original magnification ×40).

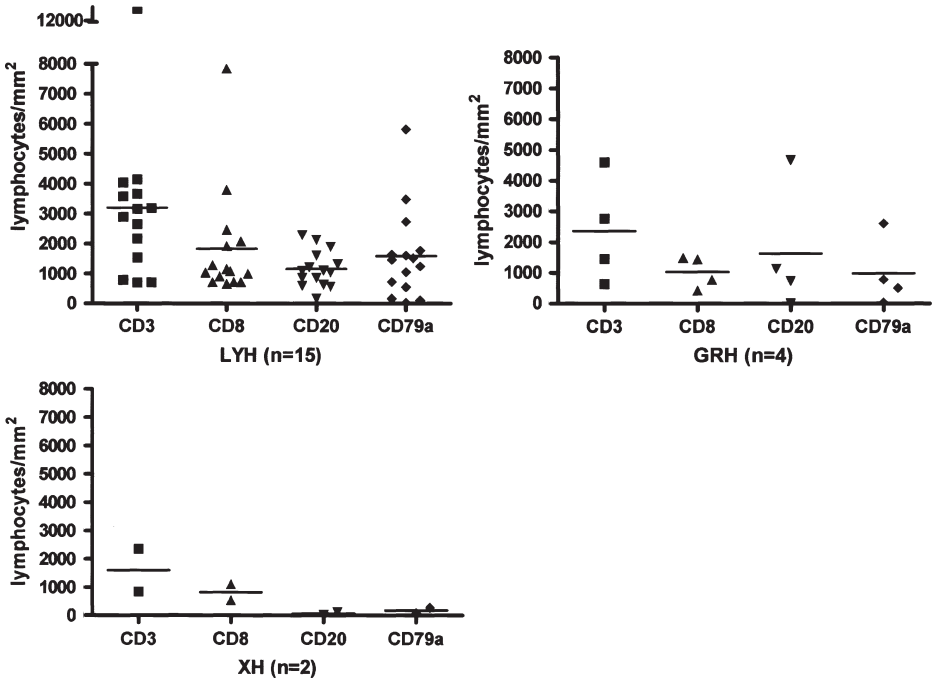

FIGURE 3. Density of lymphocytes in primary hypophysitis. LYH, GRH, and XH mainly consist of CD3+ T cells, while CD20+ B cells are rare. Horizontal bar denotes the arithmetic mean value. Outlier in LYH (CD3+) is excluded from analysis. There is no significant difference in lymphocyte distribution between LYH, GRH, and XH.

with a 100% share of all T cells. High numbers of activated CD8+ T cells (>25% positivity for granzyme B of all CD8+ T cells) were seen in 4 of 15 cases of LYH (case nos. 2, 3, 5, and 7a), and in 1 of 4 cases of GRH (case no. 18). In case nos. 3 and 5, activated CD8+ T cells dominated, showing 100% granzyme B positivity. Activated CD8+ T cells lay in close apposition to islands of preserved pituitary acinar cells, or even infiltrating them (Fig. 4). Independent of the histopathologic subtype, the increased rate of activated CD8+ T cells correlated with a shorter duration of clinical symptoms. More than 25% of activated CD8+ T cells were seen in patients with a mean duration of symptoms of 4.4 weeks, whereas no activated CD8+ cells were seen in patients with a mean duration of symptoms of 52.9 weeks. Furthermore, all samples from patients presenting with LYH in pregnancy (case nos. 2, 3, and 5) showed high numbers of activated CD8+ T cells.

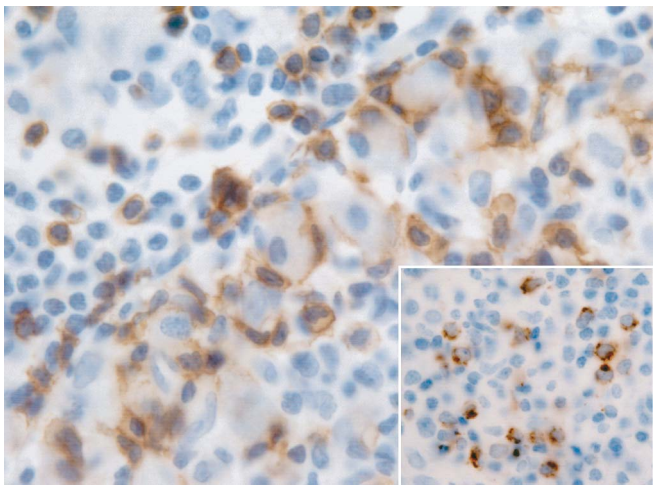

FIGURE 4. Membranous staining for CD8+ in LYH. Cytotoxic lymphocytes surround or even infiltrate islands of pituitary cells. Granular staining for granzyme B shows activated CD8+ T cells in close apposition to pituitary cells (insert) (original magnification ×100).

In none of the tissue samples analyzed was positive staining for C9neo, which recognizes the activated membrane attack complex, found.

High numbers of Ki-M1P-positive macrophages were found in every case of primary hypophysitis (Fig. 5). However, there was a wide variation in total macrophage count within the same histologic subgroup. The highest numbers of macrophages were found in XH, and macrophage numbers for LYH and GRH were comparable. Inflammatory macrophage markers were found in all of the analyzed hyophysitis samples (Fig. 6). Only low numbers of early activated macrophages positive for MRP14 and 27E10 were found in all cases. In contrast, high numbers of MRP8- and 25F9-positive macrophages, representing inflammatory macrophages late in inflammation, were detected in all cases (Fig. 6). Multinucleated giant cells in GRH cases were positive for Ki-M1P, 25F9, HC-10, and CR3/43 and did not react with antibodies against MRP8, MRP14, and 27E10 (Fig. 5). All cases, independent of their histologic subtype, showed small numbers of HC-10-positive macrophages and high numbers of HC-10-positive mononuclear cells, presumably B lymphocytes, as well as high numbers of CR3/43-positive macrophages, indicating MHC class I and II positivity, respectively.

Moreover, up-regulation of MHC class I epitopes was observed in 3 of 14 samples with preserved pituitary cells (case nos. 1, 3, and 5); and in 2 of them (case nos. 3 and 5), pituitary cells also showed strong MHC class II expression (Fig. 7). Both patients presented during pregnancy, whereas in all other primary hypophysitis as well as in control samples,

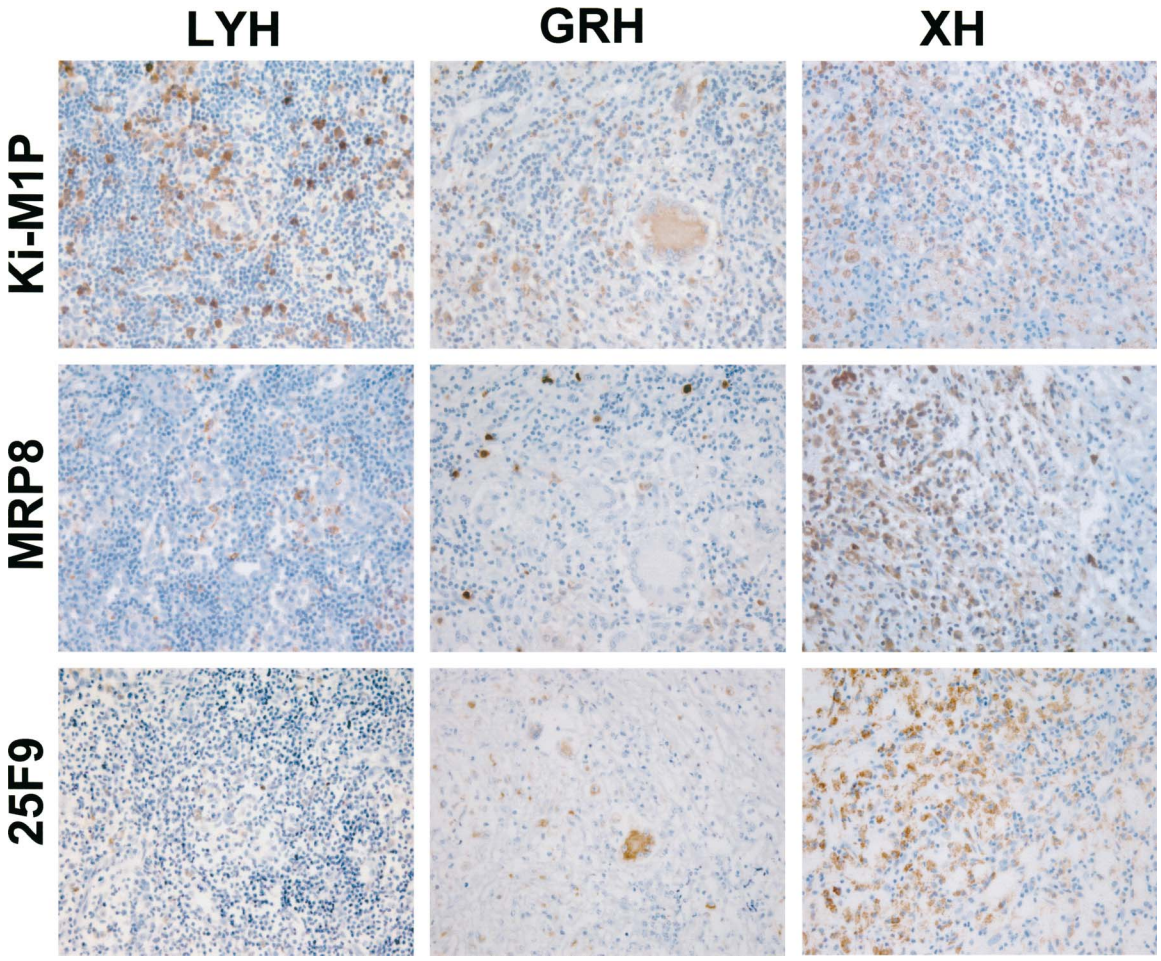

FIGURE 5. Immunohistochemical stainings for macrophage antigens. Inflammatory macrophage markers are found in all of the samples analyzed. Multinucleated giant cells in GRH cases are positive for Ki-M1P, 25F9, HC-10, and CR3/43 and do not react with antibodies against MRP8, MRP14, and 27E10. Stainings for MRP14, 27E10, HC-10, and CR3/43 not shown (original magnification ×40).

pituitary cells were only slightly positive for HC-10 and completely negative for CR3/43.

DISCUSSION

The dominance of T cells with an equal share of cytotoxic and helper T cells in primary hypophysitis infiltrates is in concordance with the composition of inflammatory infiltrates of other well-characterized endocrine autoimmune diseases, like Hashimoto's thyroiditis and juvenile diabetes mellitus.[29] The highest density of lymphocytes was observed in LYH, less in GRH, and the least infiltration by lymphocytes was seen in XH. This is due to the fact that in XH cellular infiltration consisted mainly of xanthomatous macrophages, and all GRH samples showed a massive amount of fibrosis, diminishing the number of lymphocytes. Not only for GRH, but for all analyzed cases of primary hypophysitis, the number of T cells correlated significantly with fibrosis, showing decreasing infiltration with higher amounts of fibrosis. Fibrosis in hypophysitis is proposed to be the end stage of the disease; therefore, it is thought to be a marker of the dynamics of inflammation in hypophysitis.[13] However, in our study, fibrosis did not correlate with the duration of clinical symptoms. Instead, independent of the histopathologic subtype, the amount of activated CD8+ T cells significantly correlated with shorter duration of symptoms. In addition, the highest numbers of activated CD8+ T cells were observed in patients presenting with LYH during pregnancy. In these patients, biopsy samples were taken within 8 weeks after the beginning of symptoms. Interestingly, in 2 of the 3 patients presenting during pregnancy (case nos. 3 and 5), activated CD8+ T cells lay in close apposition to preserved pituitary islets or even surrounded them, indicating direct CD8+-mediated cytotoxicity. Supporting this observation, pituitary cells in CD8+-dominated samples showed strongly up-regulated MHC class I molecules (Fig. 7). Recent evidence indicates that autoreactive CD8+ T cells contribute substantially to tissue damage in human autoimmune disorders.[23] The current view is that cross-presentation of antigens in the absence of danger signals, resembling pathogen-associated molecules, fails to give maturation signals to T cells.[31] However, de Jersey et al[10] showed that neuroendocrine self-antigens of the pituitary gland secreted in a hormone-like fashion are

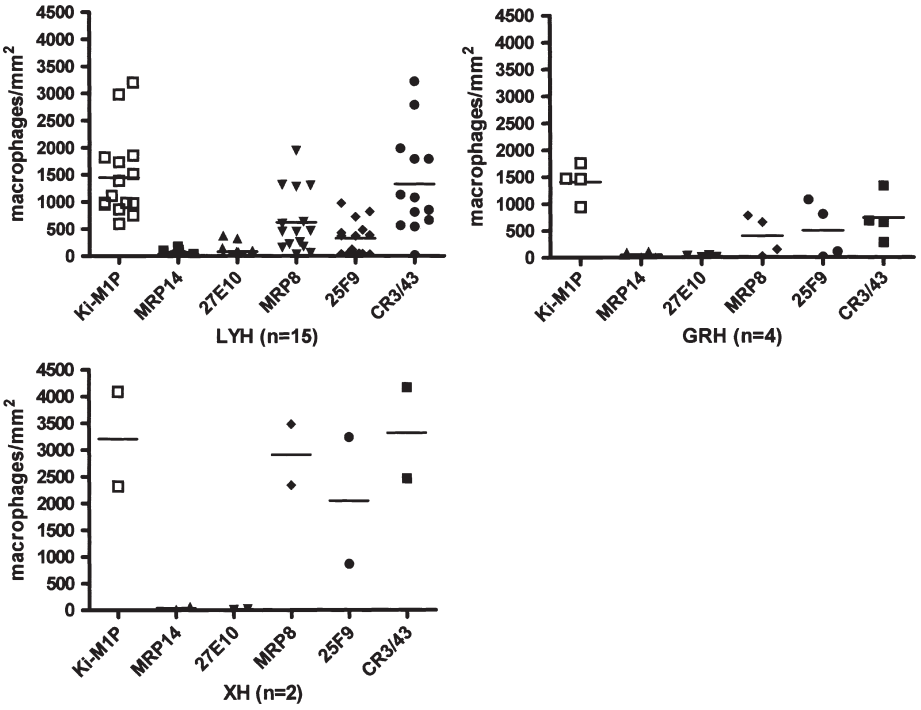

FIGURE 6. Density of macrophages in primary hypophysitis. There is a wide variation in total macrophage count within the same histologic subgroup. The highest numbers of macrophages are found in XH; macrophage numbers for LYH and GRH are comparable. Note that in all cases detectable macrophages express activation-associated antigens only from late phase of chronic inflammation (MRP 8 and 25F9), and almost no MRP 14- and 27E10-positive macrophages are found. Independent of their histologic subtypes, tremendous numbers of CR3/43 are seen.

cross-presented by dendritic cells and indeed visible for CD8+ T cells in peripheral lymphoid organs, causing autoimmune activation. In keeping with this observation, the pituitary gland might be highly susceptible for T cell-mediated autoimmune responses.

The lack of acute inflammation markers MRP14 and 27E10 on macrophages in primary hypophysitis was surprising. Even in patients with a short duration of clinical symptoms, almost all detectable macrophages expressed activation-associated antigens from only the late phase of chronic inflammation. This might be due to the fact that circulating monocytes and macrophages already lose their MRP14 and 27E10 expression a few days after infiltrating organ tissue and terminally differentiate into tissue macrophages.[22] Even more important, no macrophages and monocytes seem to be perpetually recruited from blood into inflamed pituitary. Together with the characteristic high amount of fibrosis and loss of lymphocytic cellularity in primary hypophysitis with increasing duration of clinical symptoms, this finding indicates that hypophysitis is a long-lasting but self-limiting process.

In all of our GRH cases, MRP8- and 25F9-positive mononuclear macrophages were observed, whereas mononuclear macrophages expressing MRP14 and 27E10 were not seen. According to a study by Delabie et al,[11] MRP14 expression by mononuclear macrophages in systemic granulomatous diseases is thought to be generally strong. Macrophages in granulomas of foreign body type, cat-scratch disease, and erythema nodosum strongly express MRP8, whereas MRP8 expression is weak or absent in mononuclear phagocytes of sarcoidosis and tuberculosis. Antigenic variations described here probably reflect differences in antigenic stimuli and macrophage function and therefore differentiate macrophages in GRH from phagocytes in delayed hypersensitivity-type granulomas and mononuclear phagocytes in nonhypersensitivity and nonimmunologic granulomas. Apart from the possible biologic implications, the practical diagnostic application remains disputable regarding how far MRP8 and MRP14 expression are distinct markers for differentiating GRH from systemic granulomatous diseases.

In all cases of hypophysitis, independent of their histologic subtypes, tremendous numbers of CR3/43- (ie, MHC class II) and Ki-M1P-positive cells were seen in comparison to normal pituitary controls, where numerous CR3/43-positive stellate-shaped cells, presumably dendritic cells, and only a few Ki-M1P- and CR3/43-positive macrophages were found. Macrophages acquire MHC class II positivity after stimulation by pathogens or cytokines released by CD4 T_H1 cells. The high numbers of MHC class II-positive macrophages in LYH, GRH, and XH indicate a CD4+-mediated immune response, where T_{H1} cells lead to macrophage recruitment and activation.

Moreover, the high expression of the MHC class II epitope on macrophages in hypophysitis is probably also important for understanding the initial phase of hypophysitis. The production of autoantibodies against pituitary hormones or pituitary tissue and in particular the expansion of T_{H1} and T_{H2} as well of cytotoxic T cells in primary hypophysitis requires an initial presentation of the relevant autoantigens to the immune system by professional antigen-presenting cells, eg, macrophages. In addition, in 2 of the cases analyzed presenting during pregnancy, strong immunoreactivity of pituitary cells for MHC class II was observed. Aberrant expression of MHC class II molecules is seen on thyreocytes in patients with Grave's disease, on pancreatic β cells in patients with recent onset of juvenile diabetes mellitus,[16] on synovial cells in rheumatoid arthritis, and on endothelial cells in systemic lupus erythematosus.[36] Several studies have suggested that the MHC

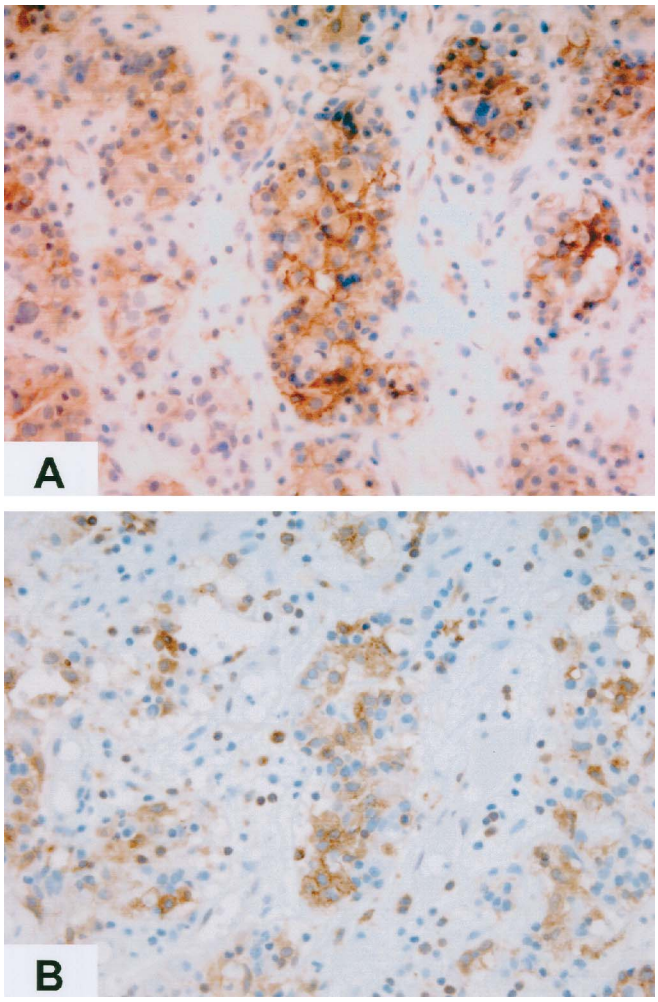

FIGURE 7. Preserved islands of pituitary cells in LYH. A, Overexpression of MHC class I molecules on pituitary acinar cells (membranous staining for HC-10). B, Aberrant expression of MHC class II molecules on pituitary acinar cells (membranous staining for CR3/43) (original magnification ×40).

class II antigen expression is secondary to lymphocyte infiltration and organ destruction.[18,24] However, the demonstration of antibodies to alpha- and gamma-enolase, an antigen shared by pituitary cells and cells of the "diffuse endocrine system" (APUD cells) and even more, by the placenta,[2,9,27,28,32,33] supports the hypothesis that the aberrant expression of MHC class II antigens on pituitary cells observed in this study might result in the activation of autoreactive CD4+ lymphocytes and therefore might play a leading role in the initiation of primary hypophysitis.

In summary, the subtypes of primary hypophysitis, although heterogeneous in their histologic appearance and age distribution, exhibit a similar if not identical immunohistologic profile. Taking all the observations in this study together, it is strongly suggested that direct T cell-mediated cytotoxicity through CD8+ T cells with the initial help of CD4+ T cells is pivotal in the pathogenesis of hypophysitis, implicating a target autoantigen from pituitary cells.

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