Hyaline Droplets in Kupffer Cells

A Novel Diagnostic Clue for Autoimmune Hepatitis

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Abstract: Pediatric autoimmune hepatitis (AIH) is relatively common and has a characteristic but relatively nonspecific histopathology with a usually prominent lymphoplasmacytic infiltrate. Herein, we describe for the first time the presence of characteristic hyaline droplets in the cytoplasm of Kupffer cells on routine hematoxylin and eosin (H&E) sections in AIH. The medical records and pathologic material over a 20-year period (1992 to 2012) were reviewed from children with AIH (n = 30), hepatitis B virus (n = 30), and hepatitis C virus (n = 30) from the pathology files at Boston Children's Hospital. All children had percutaneous needle liver biopsies. We reviewed sections stained with H&E, PAS, and PAS with diastase for the presence of hyaline droplets in all 90 biopsies. We also performed immunohistochemical analysis for IgG, IgA, and IgD in 6 biopsies with AIH. Hyaline droplets were identified in Kupffer cells throughout the lobules in 15 of 30 biopsies (easily found in 13 and rare in 2); conversely, no droplets were identified in 15. Droplets were identified in 10 AIH type 1 biopsies, 1 in AIH type 2, 3 in overlap syndrome, and 1 in unclassified. Serum IgG levels, when available, were correlated with biopsy findings. Seventeen patients had serum IgG levels available for review. The average IgG level in patients without droplets in their biopsies was 1364 mg/dL, in contrast to 3424 mg/dL in patients with droplets ($P = 0.021$). Immunohistochemical analysis performed in 6 biopsies revealed that droplets were nearly always positive for IgG, occasionally for IgA, and rarely for IgD. None of the biopsies in patients with hepatitis C contained hyaline droplets. One biopsy of a patient with hepatitis B revealed hyaline droplets; this biopsy had an unusually prominent plasmacytic infiltrate, and the patient was found to have an elevated IgG serum level and antibodies to smooth muscle actin. As far as we are aware, hyaline droplets in Kupffer cells on routine H&E sections have never been described. They should be distinguished from the nonspecific granular lysosomal structures frequently found in Kupffer cells in a variety of chronic liver diseases and from erythrophagocytosis. Hyaline droplets may occur in AIH regardless of the type and correlate with a > 2-fold increase in serum level of IgG as compared with patients without droplets in their biopsies. Identification of hyaline droplets in Kupffer cells provides a useful diagnostic clue to distinguish AIH from other forms of chronic hepatitis.

Key Words: immunoglobulins, Kupffer cell, Russell body

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Autoimmune hepatitis (AIH) is a chronic hepatitis associated with hypergammaglobulinemia, hepatocyte-directed autoantibodies, and is, in most instances, responsive to immunosuppressive therapy. The International Autoimmune Hepatitis Group (IAIHG) requires a liver biopsy for definitive diagnosis, preferentially before the start of immunosuppressive therapy. The IAIHG defines the histologic features of AIH as characteristically consisting of an interface hepatitis with a predominantly lymphoplasmacytic infiltrate, rosetting of hepatocytes, and no biliary changes or features typical of another etiology; however, no pathognomonic criteria for AIH exist.[1]

Characteristic autoantibodies in the serum of patients suspected of having AIH are central to diagnosis and define the subtype of illness. Therefore, whenever AIH is in the differential diagnosis, testing for antinuclear antibody (ANA), smooth muscle antibody (SMA), and liver-kidney microsome 1 antibody (LKM1) is often performed. Those patients with a positive ANA and/or SMA are designated type 1 AIH, whereas those with LKM1 and/or antiliver cytosol type 1 antibody (anti-LC1) are designated type 2 AIH.[2–4]

Kupffer cell hyperplasia has been a noted feature in AIH histology, although what role these cells play in the disease process in unknown.[2] Immunoglobulin deposition was demonstrated by immunofluorescence techniques within the cytoplasm of Kupffer cells in AIH over 50 years ago.[5,6] However, these reports did not describe any characteristic inclusions in the Kupffer cells on hematoxylin and eosin (H&E)-stained sections or any other special stains or microscopic techniques. Comparison of the number of cells containing immunoglobulins with the respective serum immunoglobulin levels also failed to show an overall correlation.[6]

We are reporting for the first time, as far as we are aware, distinctive hyaline droplets in Kupffer cells in AIH visible on routine H&E-stained section. These droplets

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provide a new diagnostic clue in the diagnosis of AIH helping to differentiate AIH from other forms of chronic hepatitis.

MATERIALS AND METHODS

Review of Clinical and Histopathologic Material

A retrospective study was conducted. The medical records and pathologic material were reviewed from 30 patients with AIH, 30 with hepatitis B virus infection, and 30 with hepatitis C virus infection diagnosed over a 20-year period (1993 to 2013). The medical records were reviewed for laboratory data, including serum IgG levels, autoantibody titers, and viral serologies. Clinical notes were also reviewed to confirm the liver biopsy diagnosis, as well as for evidence that other mimics of AIH had been excluded. All patients had percutaneous needle liver biopsies.

Patients with AIH were subtyped depending on their clinical information, laboratory data, and histopathologic features into 4 groups: type 1 AIH, type 2 AIH, overlap syndrome of AIH with primary sclerosing cholangitis, and unclassified, representing those patients with AIH who did not have autoantibody titers available for review at Boston Children's Hospital.

Serum IgG levels available in the medical records at Boston Children's were correlated with the presence or absence of hyaline droplets in the liver biopsies. A Student 2-tailed *T* test was utilized for statistical comparison analysis, with a *P*-value of <0.05 representing statistical significance.

Formalin-fixed paraffin-embedded sections stained with H&E, periodic acid Schiff (PAS), and PAS post-diastase (PASD) from the 90 liver biopsies were reviewed for the presence of hyaline droplets within the cytoplasm of Kupffer cells. The number of droplets present in the biopsies were semiquantitated and ascribed a numerical score as 0 designating that no droplets were identified, 1+ designating rare droplets (1 to 2/high-power fields), and 2+ designating that droplets were easily identifiable (≥ 3/high-power fields).

Immunohistochemical Staining

Immunohistochemical staining was performed on formalin-fixed paraffin-embedded sections in 6 liver biopsies utilizing antibodies against IgG (Cell Marque, Rocklin, CA), IgA (Dako, Carpinteria, CA), and IgD (Leica Biosystems, Buffalo Grove, IL) in 6 selected AIH biopsies. Staining present only in the characteristic droplets in the Kupffer cells was analyzed. The intensity of staining was semiquantitated on a scale from 0, which represented no appreciable staining of droplets, to 3+, which represented diffuse dark staining of droplets. Immunohistochemical staining for CD68 was also performed in 1 biopsy of AIH. With all stains, a standard technique using a peroxidase-conjugated streptavidin biotin was utilized according to the manufacturer's protocols. Appropriate control analyses were simultaneously performed for all specimens.

RESULTS

Clinical Information

The age and sex of the patients with AIH and the subtype of AIH are shown in Table 1. For the patients with hepatitis C, the average age was 15 years with a range of 2 to 30 and included 17 male and 13 female individuals. For the patients with hepatitis B, the average age was 13 years with a range of 2 to 23 and included 19 male and 11 female individuals. For the patients with AIH, the average age was 15 years with a range of 6 to 23 and included 16 male and 14 female individuals. For the patients with AIH, 17 had type 1, 2 had type 2, 7 had AIH-PSC overlap syndrome, and 4 were unclassified.

Histopathologic Findings

Hyaline droplets were identified in the Kupffer cells of 15 of the 30 (50%) liver biopsy specimens in patients with AIH, with 13 of these biopsies demonstrating 2+ droplets and 2 biopsies demonstrating 1+ droplets (Figs. 1, 2) Conversely, no droplets were identified in the remaining 15 patients with AIH. The droplets were well-circumscribed bodies with a glassy appearance within the cytoplasm of the Kupffer cells throughout the lobules (Fig. 3). These droplets were PAS positive and diastase resistant (Fig. 3B). Droplets were identified in a spectrum of biopsies with AIH features, including those with mild

TABLE 1. Summary, Patients With AIH

Case No.	Subtype	Sex	Age	Droplets	IgG Levels
1	OL	M	11	2+ droplets	2160
2	Type 1	F	12	2+ droplets	NA
3	Type 1	F	16	2+ droplets	8356
4	OL	M	20	1+ droplets	NA
5	Type 1	F	12	2+ droplets	NA
6	Type 1	M	13	2+ droplets	2093
7	Type 1	F	6	2+ droplets	2650
8	Type 2	M	13	2+ droplets	1435
9	Type 1	F	11	2+ droplets	5334
10	Type 1	M	18	2+ droplets	NA
11	OL	M	17	2+ droplets	3232
12	Type 1	F	20	1+ droplets	NA
13	Unclassified	M	11	2+ droplets	2314
14	Type 1	F	9	2+ droplets	4130
15	Type 1	F	13	2+ droplets	2538
16	Unclassified	F	19	NDI	799
17	OL	M	16	NDI	2161
18	Type 1	F	15	NDI	1516
19	Type 1	M	19	NDI	NA
20	Type 1	M	23	NDI	NA
21	OL	M	20	NDI	NA
22	Type 1	F	13	NDI	NA
23	Type 1	F	19	NDI	NA
24	Type 2	M	17	NDI	1300
25	Type 1	M	18	NDI	1177
26	Unclassified	M	10	NDI	NA
27	OL	F	16	NDI	NA
28	OL	M	15	NDI	1265
29	Unclassified	F	18	NDI	1333
30	Type 1	M	10	NDI	NA

NA indicates not available; NDI, no droplets identified; OL, overlap syndrome.

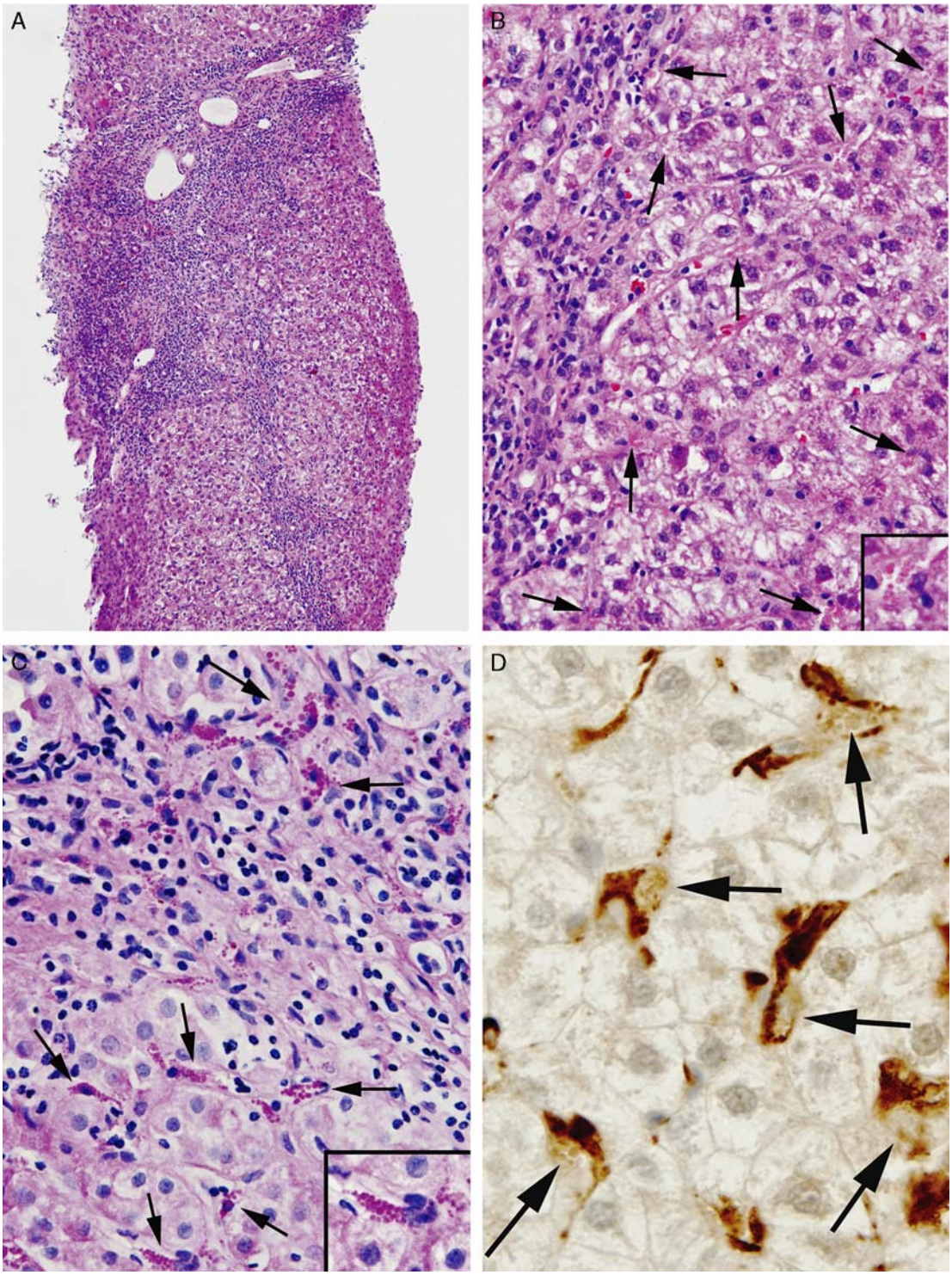

FIGURE 1. A, Liver biopsy with AIH characterized by marked portal lymphoplasmacytic infiltrate spilling into the lobules and blurring the limiting plates. B, Diffuse hepatocellular ballooning and occasional apoptotic hepatocytes are shown at higher magnification. Arrows indicate enlarged Kupffer cells with cytoplasmic hyaline globules scattered throughout the lobule which are seen in the inset at higher magnification. C, PAS-positive diastase-resistant cytoplasmic hyaline globules in Kupffer cells are indicated by arrows and are seen in the inset at higher magnification. D, CD68 immunohistochemistry shows Kupffer cells with clusters of unstained hyaline globules surrounded by positively stained lysosomal structures.

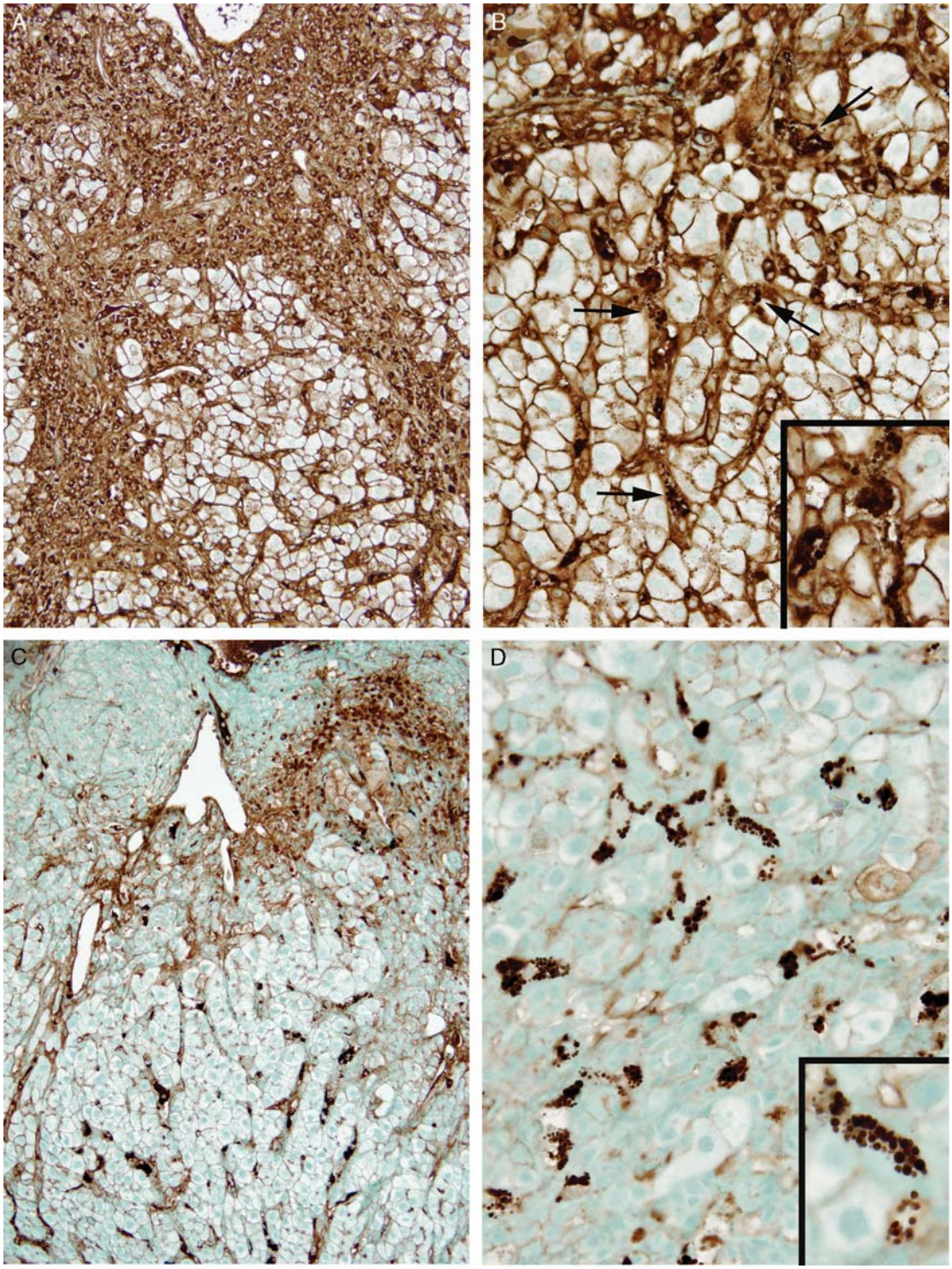

FIGURE 2. A, IgG immunohistochemistry showing prominent staining of portal tracts and septae. B, IgG-positive cytoplasmic globules in Kupffer cells are indicated by arrows and can be seen in the inset at higher magnification. C, IgA immunohistochemical stain showing portal and sinusoidal positivity. D, At higher magnification, IgA-positive cytoplasmic globules are seen in Kupffer cells scattered throughout the lobule and in the inset at higher magnification.

lymphoplasmacytic infiltrate and hepatocellular damage to those biopsies with a dense lymphoplasmacytic infiltrate and submassive hepatic necrosis. Neither the presence nor number of hyaline globules in Kupffer cells correlated with the degree of inflammation, number of plasma cells, or grade or stage of the chronic hepatitis.

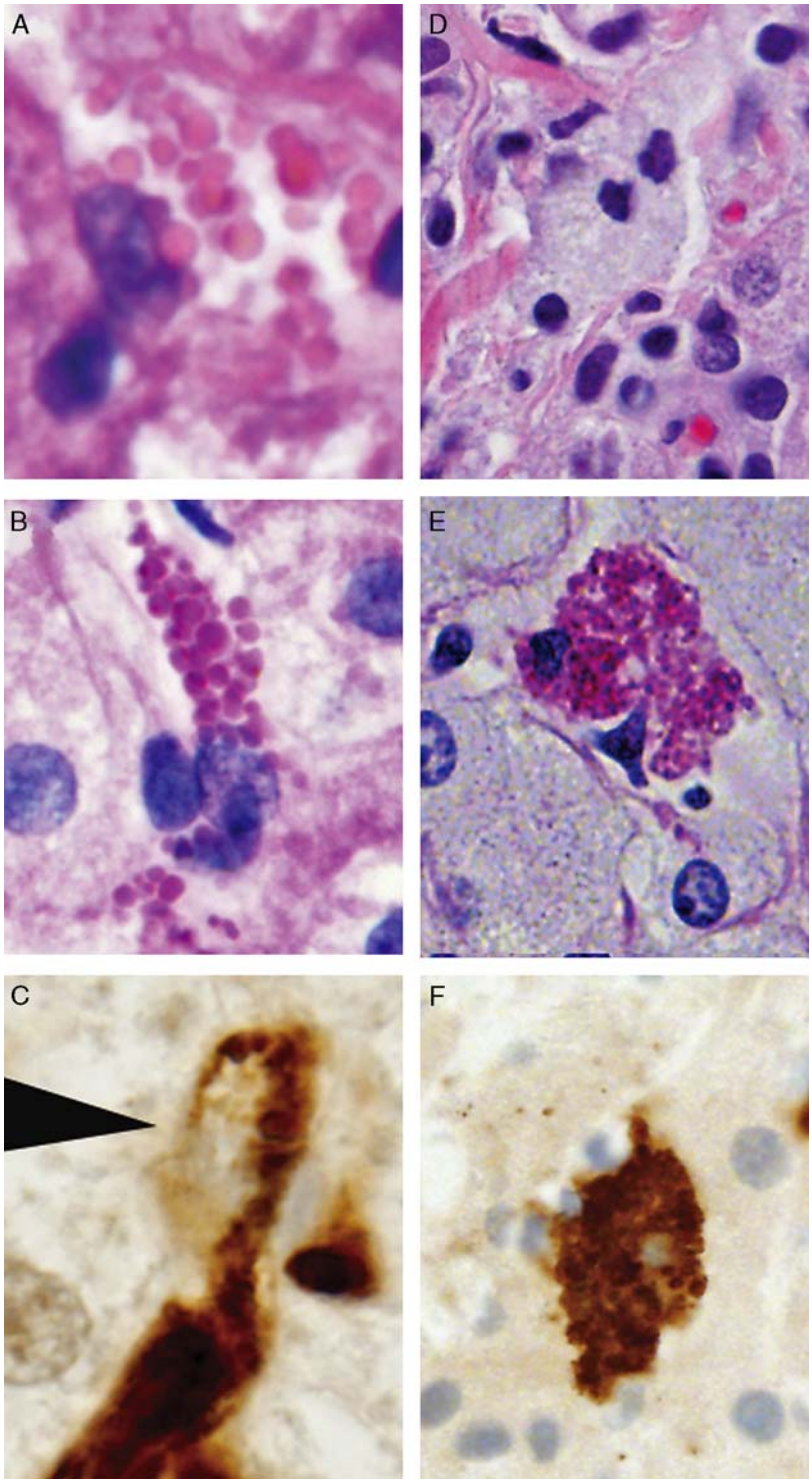

FIGURE 3. A, Kupffer cell with numerous hyaline lobules with smooth glassy appearance. Droplets are round and separated from each other. B, Numerous cytoplasmic globules in a Kupffer cell showing intense PAS diastase-resistant positivity. C, A cluster of CD68 immune-negative globules (arrowhead) are surrounded by numerous CD68 immunoreactive lysosomal structures. D, Kupffer cells with ceroid pigment with a granular gray appearance. E, Ceroid pigment-laden Kupffer cell showing confluent PAS positive, diastase-resistant lysosomal structures with a granular appearance. F, Ceroid pigment-laden Kupffer cell with cytoplasmic lysosomal structures intensely immunoreactive for CD68.

Globules were not found more often in proximity to acidophilic bodies or foci of hepatocyte necrosis.

In the 30 patients with hepatitis C, none of their biopsies contained hyaline droplets. In the 30 patients with hepatitis B, 1 biopsy revealed hyaline droplets. This biopsy had an unusually prominent plasmacytic infiltrate.

Immunohistochemistry

All 6 biopsies in patients with AIH in which immunohistochemistry was performed revealed positivity for IgG and IgA in the droplets ranging in intensity from 1+ to 3+ (Fig. 2). The intensity of staining appeared to have an inverse relationship, with those biopsies with greater IgA staining intensity having less IgG staining intensity and vice versa. Only 1 biopsy demonstrated a similar staining intensity for IgG and IgA. None of the biopsies demonstrated Kupffer cell droplet staining for IgD. However, all 5 biopsies showed IgD staining within plasma cells. In the single biopsy stained for CD68, the hyaline droplets were negative (Figs. 1D, 3C). This contrasted with the phagolysosome granules in the Kupffer cells, which stained strongly positive.

Correlation With Serum IgG

Upon review of the clinical records, 17 of the 30 patients with AIH (57%) had serum IgG levels available for review (Table 1). The average IgG level in patients without droplets in their liver biopsies was 1364 mg/dL, in contrast to 3424 mg/dL in patients with droplets ($P = 0.021$). The normal range for IgG at Boston Children's Hospital is 639 to 1344 mg/dL. Other immunoglobulin levels were not compared because of limited clinical data. The patient with chronic hepatitis B virus infection who had hyaline droplets in the liver biopsy revealed an IgG serum level of 2378 mg/dL; this patient was also found to have antibodies to smooth muscle actin.

DISCUSSION

Liver biopsy is an integral part of the diagnosis of AIH. Nonetheless, the histopathology of AIH is characteristic but not specific. The histologic features of AIH consist of a chronic hepatitis pattern with a predominantly lymphoplasmacytic portal infiltrate, often with plasma cell clustering and interface and lobular necroinflammatory activity. The histopathology, however, can vary widely depending on the grade and stage of the process. During periods of quiescence, portal inflammation may be the sole histologic abnormality. With increased disease activity, lobular hepatocellular damage is identified with hepatocyte ballooning degeneration, pseudoacinar transformation, extensive necroinflammatory activity and variable parenchymal collapse, including submassive and massive hepatic necrosis.[2,7] Generally, no biliary changes or features typical of another etiology should be present for the diagnosis of AIH.[1,8] The chronic hepatitis pattern of injury seen in AIH overlaps with chronic hepatitis B and C viral infections and may be histologically indistinguishable.[7] Although plasma cells are more commonly identified in AIH, they can also be identified, although to a lesser extent, in hepatitis B and C. Lobular hepatocellular damage may also be seen in viral hepatitis, particularly in hepatitis B, with ballooning and pseudoacinar transformation of hepatocytes. In acute viral hepatitis, these changes may be more pronounced and can include massive hepatic necrosis. Besides the clinical serological differences, on histology, secondary differentiating characteristics include ground-glass hepatocytes resulting from cytoplasmic accumulation of hepatitis B surface antigen within smooth endoplasmic reticulum in chronic hepatitis B and focal aggregates of hepatocytes with macrovesicular steatosis in hepatitis C infection. Portal lymphoid aggregates or follicles may occur in chronic hepatitis B and C as well as in AIH.

Hyaline droplets in Kupffer cells on routine H&E sections have never been described in AIH, to the best of our knowledge. Identification of these droplets provides a useful diagnostic clue to distinguish AIH from other forms of chronic hepatitis, as only 1 case of the 60 viral hepatitis cases examined demonstrated droplets. Of note, in this viral hepatitis case, the biopsy contained an unusually prominent lymphoplasmacytic infiltrate, and the patient was found to have an elevated serum IgG level as well as antibodies against smooth muscle actin, raising the possibility of an autoimmune component to his liver disease.

As the histopathology of AIH has been well described for many years, it seems surprising that the existence of hyaline droplets in Kupffer cells in patients with this disorder, as described herein, has escaped recognition for such a long time. This lack of awareness, however, may possibly be explained by the morphologic similarity of hyaline droplets to heterophagolysosomes. Heterophagolysosomes are ubiquitous in the cytoplasm of Kupffer cells in a variety of chronic liver disorders and are thought to represent phagocytosed waste material resulting from necrosis and increased cell turnover. The hyaline droplets described herein can be distinguished from heterophagolysosomes by a number of histologic criteria. Whereas hyaline droplets on H&E sections have a round sharply circumscribed and glassy appearance with a homogenous pale-pink color, lysosomal structures are granular, ill defined, and have a white-gray-bluish color. Hyaline droplets are separated from each other and do not coalesce; their morphology is identical to the Russell bodies of plasma cells. By contrast, heterophagolysosomes have irregular outlines and tend to coalesce with each other forming ill-defined groups. They are identical to the contents of ceroid-laden macrophages. These morphologic differences between hyaline droplets and heterophagolysosomes are highlighted by PAS staining. Although both types of inclusions are PAS positive diastase resistant, their detectability and morphologic characteristics are highlighted by these special stains. In addition, by immunohistochemistry, hyaline droplets are negative for CD68, a marker of macrophages, whereas heterophagolysosomes are strongly pos-

TABLE 2. Summary, Inclusions in Kupffer Cells

Hyaline Droplets	Lysosomal Granules	Erythrophagocytosis
Pale pink on H&E stain	Gray-white on H&E stain	Pink-Red on H&E stain
PAS positive, diastase resistant; globular with a well-circumscribed, waxy appearance	PAS positive, diastase resistant; granular with a finer, less well-circumscribed appearance	PAS negative
Similar to a Russell body in a plasma cell	Similar to a ceroid-laden macrophages	Red blood cell
CD68 negative	CD68 positive	CD68 negative

itive. Another possible explanation for this lack of awareness of the existence of hyaline droplets is their close resemblance to red blood cells, and perhaps they have been misinterpreted in the past as erythrophagocytosis by Kupffer cells. Hyaline droplets can be distinguished from erythrocytes by their variable size, often smaller than erythrocytes, and by their intense PAS-positive and PASD-positive staining. Histologic differences between these 3 types of cytoplasmic inclusions are summarized in Table 2.

Correlation of the presence or absence of droplets in the liver biopsies to the patient's clinical data reveals that droplets are found in both type 1 and type 2 AIH, in overlap syndrome of AIH with primary sclerosing cholangitis, and in a single patient with hepatitis B. In addition, the presence of droplets correlates with a statistically significant >2-fold increase in serum level of IgG as compared with patients without droplets ($P = 0.021$)

Kupffer cells are nonparenchymal cells that account for approximately 15% of the total cell population in the liver and 80% to 90% of the tissue-resident macrophages in the whole body.[9] Because of their sinusoidal localization, Kupffer cells scavenge a number of particulate materials from the portal circulation, including senescent red blood cells, immune complexes, and gut-derived bacterial products.[9] Understanding of the Kupffer cells' role in the pathogenesis of AIH is limited. In general, Kupffer cells express a number of receptors including scavenger receptors like SR-A, as well as FC γ and α receptors.[10] Hepatic SR-A expression dramatically increases in patients with AIH and viral hepatitis.[11] Studies emerging from the field of tumor immunology recently showed that SR-A acts as a suppressor of antigen presentation, T-cell activation, and antitumor immunity by modulating the intrinsic immunogenicity of antigen-presenting cells.[11] The IgA receptor on Kupffer cells includes the myeloid-specific IgA Fc receptor (FcRI or CD89). Cells that express these receptors play a regulatory role, either by degrading IgA antibody complexes or by recycling serum IgA to achieve serum homeostasis, possibly depending on receptor clustering size.[12] Literature investigating the role of IgA in AIH is limited, but studies have shown an upregulation of IL-21 in patients with AIH.[13] IL-21 is predominately produced by follicular helper T cells and regulates humoral responses. The concentration of IL-21 correlates with the levels of serum IgA, and IL-21 is known to increase the production of IgG, IgM, and IgA.[13] These findings suggest that excess immunoglobulins produced by plasma cells in AIH are ultimately taken up and recycled by Kupffer cells.

In summary, hyaline droplets in Kupffer cells provide a useful histologic clue in the diagnosis of AIH. It is seen in both types of AIH as well as in the overlap syndromes of AIH/primary sclerosing cholangitis. Hyaline droplets were noted in cases with varying amounts of lymphoplasmacytic infiltrate and hepatocellular damage, including biopsies with a minor lymphoplasmacytic component. The hyaline droplets may be especially useful when available diagnostic tissue is limited, as is often the case in needle core biopsies. Hyaline droplets provide a useful histologic feature to differentiate AIH from other forms of chronic hepatitis. The presence or absence of droplets also correlates with the serum levels of IgG.

REFERENCES

1. Alvarez F, Berg PA, Bianchi FB, et al. International Autoimmune Hepatitis Group. Report: review of criteria for diagnosis of autoimmune hepatitis. *J Hepatol*. 1999;31:929–938.
2. Burt AD, Portmann BC, Ferrell LD. *MacSween's Pathology of the Liver*. 6th edn. Philadelphia, PA: Churchill Livingstone Elsevier; 2011.
3. Gossard AA, Lindor KD. Autoimmune hepatitis: a review. *J Gastroenterol*. 2012;47:498–503.
4. Mieli-Vergani G, Vergani D. Autoimmune hepatitis in children: what is different from adult AIH? *Semin Liver Dis*. 2009;29:297–306.
5. Cohen S, Ohta G, Singer EJ, et al. Immunocytochemical study of gamma globulin in liver in hepatitis and postnecrotic cirrhosis. *J Exp Med*. 1960;111:285–294.
6. Hadziyannis S, Feizi T, Scheuer PJ, et al. Immunoglobulin-containing cells in the liver. *Clin Exp Immunol*. 1969;5:499–514.
7. Saxena A. *Practical Hepatic Pathology: A Diagnostic Approach*. Philadelphia, PA: Elsevier Saunders; 2011.
8. Johnson PJ, McFarlane IG. Meeting report: International Autoimmune Hepatitis Group. *Hepatology*. 1993;18:998–1005.
9. Sitia G, Iannacone M, Aiolfi R, et al. Kupffer cells hasten resolution of liver immunopathology in mouse models of viral hepatitis. *PLoS Pathog*. 2011;7:e1002061.
10. Lovdal T, Andersen E, Brech A, et al. Fc receptor mediated endocytosis of small soluble immunoglobulin G immune complexes in Kupffer and endothelial cells from rat liver. *J Cell Sci*. 2000;113(pt 18):3255–3266.
11. Zuo D, Yu X, Guo C, et al. Scavenger receptor A restrains T-cell activation and protects against concanavalin A-induced hepatic injury. *Hepatology*. 2013;57:228–238.
12. Monteiro RC, Van De Winkel JG. IgA Fc receptors. *Annu Rev Immunol*. 2003;21:177–204.
13. Ma L, Qin J, Ji H, et al. Tfh and plasma cells are correlated with hypergammaglobulinaemia in patients with autoimmune hepatitis. *Liver Int*. 2013;34:405–415.