sexual reproduction; and in certain auxiliary attributes (see Tables 1, 2, and 3).

In addition, *H. lobata* also exhibits distinct differences from *H. rugosa*: in the size and shape of motile cells; in chloroplast structure; in the size of immobile cells; and in certain auxiliary attributes (see Tables 1, 2, and 3).

Since the organisms herein described demonstrate several well-defined morphologic and physiologic differences, they are designated as separate taxa of the genus *Heterochlamydomonas.*

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SPLENIC FIBRINOID NECROSIS IN MOUSE RADIATION CHIMERAS WITH SECONDARY DISEASE*

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ABSTRACT

Fibrinoid necrosis was observed in three strains of mice following irradiation and transplantation of parental spleen or allogeneic bone marrow. Radiation alone or injection of hematopoietic cells without radiation did not induce the lesion. The presence of fibrin within areas of necrosis was confirmed with five commonly used histological stains. The morphological and staining characteristics of these lesions were identical with those generally described in fibrinoid necrosis.

Irradiated mice that have received allogeneic bone marrow, parental spleen, or parental bone marrow cells develop a clinical syndrome called "secondary disease." It is also known as "foreign spleen or bone marrow reaction" and appears within 2 weeks after cell injection. Considered to be an immunologic reaction, the disease

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is characterized histologically by extensive lesions in the lymphatic tissues (lymph nodes, white pulp of spleen, and Peyer's patches), along with associated atrophy of thymus, bone marrow, red pulp of spleen, and other organs. In the lymphatic tissues there is a marked early proliferation of a granulomatous type followed by varying degrees of hemorrhage and, if the mouse survives, necrosis, fibrosis, and ultimate repair. [1, 2] In some mice undergoing the reaction, fibrinoid necrosis occurs in the involved lymphatic tissues and bone marrow.[1, 2] Neuman[3] originally described fibrinoid necrosis as a vascular or interstitial change in tissue characterized by the presence of an acidophilic, homogeneous and refractile material with some of the staining properties of fibrin. A diagnosis of fibrinoid is usually made first on hematoxylin- and eosin-stained sections of abnormal mouse tissue. Without special staining, however, fibrinoid necrosis may be confused with fibrin, amyloid, hyalinization, and degenerative processes such as those occuring in glomerulosclerosis. This work is an attempt to determine the incidence and to study the histochemical nature of the fibrinoid lesions in the spleen accompanying secondary disease.

Materials and Methods

Young adult mice were irradiated with 300-950 rads of 250-kvp X-rays to the whole body and injected soon thereafter with $\sim$15 X 10^6 parental spleen cells intraperitoneally, parental bone marrow cells intravenously, or allogeneic bone marrow cells intravenously (Table I). Further details of irradiation and cell injection have been presented elsewhere.[1, 2] After irradiation, mice were killed at intervals covering 30 days. Spleens were examined histologically, and the presence and severity of fibrinoid necrosis were noted. A lesion was considered mild when one patch or a few small patches of fibrinoid necrosis were found around the edge of the lymphatic nodule, moderate when larger and more numerous patches occurred, and severe when patches coalesced to involve 20 percent or more of the nodule. From the large number of mice necropsied, a few selected as furnishing good examples of fibrinoid necrosis were studied histochemically. These included mice with spleen lesions from strains (C57BL X 101)F_1, (RFM X AKR)F_1, and (101 X C3H)F_1. Sections of spleen were fixed in Zenker-formol, processed through graded alcohols and toluene, and impregnated with Paraplast (56-58°C) in an automatic tissue processor. Sections 5μ thick from the control and experimental groups were stained with a variety of stains: Harris's hematoxylin and alcoholic eosin (H & E); for fibrin-phosphotungstic acid hematoxylin (PTAH) and Weigert's crystal violet,[4] Picro-Gomori trichrome and periodic acid Schiff (PAS),[6] and PAS plus orange G;[6] for connective tissue—Mallory's aniline blue-orange G;[4] for mucopolysaccharides—Hale's colloidal iron,[7] alcian blue (0.1 percent acetic acid, pH 2.5), and toluidine blue (0.25 percent aqueous solution); for amyloid—crystal violet and congo red;[4] and for reticulum—silver reticular (Foot-Bielschowsky).[8]

Control sections for fibrin were obtained from intraperitoneal blood clots in Sprague-Dawley rats injected intraperitoneally with 10 ml of whole blood from animals of the same strain. The rats were killed 2 to 9 days after injection; the clots were fixed in Zenker-formol and in 10 percent formaldehyde with dipyridinium chloride added to preserve acid mucopolysaccharides. Additional sections of tissues containing amyloid and sections of kidney from mice with age-induced as well as leukemia-associated glomerulosclerosis were compared for staining reactions.

Results

We found injection of parental-strain or allogeneic cells into mice after irradiation to be regulary associated with development of secondary disease of varying severity in several strains of mice. The numbers of mice showing fibrinoid necrosis in reactive areas of spleen white pulp are indicated in Tables I and II. Occurrence of fibrinoid necrosis was sporadic; in fact, it did not appear in most mice with severe secondary disease. Necrotic areas were observed in lymph nodes and bone marrow as well as in the spleen. The lesion appeared most frequently ($\sim$1 of 3) in irradiated mice injected with allogeneic bone marrow, in $\sim$ 1 of 10 injected with parental spleen cells, and in $\sim$1 of 20 injected with parental bone marrow cells (Table I). However, there were marked donor-recipient strain combination differences in the incidence of fibrinoid necrosis (Table II). In some strains, donor cells of one parental type were effective in inducing fibrinoid necrosis, whereas those of the other parental type were not.

TABLE I. The relationship of splenic fibrinoid necrosis to radiation dose and types of cells injected in pooled mice of all strains.

Radiation Dose (rads)	Type of Cells Injected	Fibrinoid Necrosis: Frequency	Number and Severity: Mild	Moderate	Severe
300–900	None	0/91			
0	Parental spleen or bone marrow	0/30			
400–900	Isogeneic spleen or bone marrow	2/32	2		
900	Parental bone marrow	6/114	2	3	1
400–900	Parental spleen	44/402	21	6	17
900–950	Allogeneic bone marrow	31/94	1	19	11

Preliminary study of the splenic lesion was made with hematoxylin- and eosin-stained sections. Spleens from (C57BL X 101)F_1 mice, that, 16 days previously, had received parental spleen cells contained reduced numbers of lymphocytes and an accumulation of fibrinoid material surrounding the periphery of the central arteriole of the germinal center (Fig. 1), The lesion appeared to be more severe in some spleens. (Fig. 2), with extensive hemorrhage in the lesion extending into adjacent red and white pulp; in some animals, cellular elements disappeared entirely. In those mice surviving these changes, fibrosis was the ultimate result.

TABLE II. Frequency of fibrinoid necrosis in spleens of irradiated* mice receiving spleen or bone marrow cell injections in various donor recipient strain combinations.

Strain: Recipient	Strain: Donor	Type of Cells Injected	Number Examined**	Fibrinoid Necrosis: Incidence (%)	Number With: Mild	Moderate	Severe
(C57BL X 101)F_1	C57BL	Bone marrow	19	0			
	101	Bone marrow	24	17	1	2	1
	C57BL	Spleen	79	8	7	3	
	101	Spleen	103	16	11	1	5
(RFM X AKR)F_1	AKR or RFM	Bone marrow	11	0			
	AKR	Spleen	26	50	3		10
	RFM	Spleen	23	9			2
(101 X C3H)F_1	101	Bone marrow	27	7	1	1	
	C3H	Bone marrow	19	0			
	(C57BL/6 X DBA/2)F_1	Bone marrow	82	32		18	8
	101	Spleen	73	0			
	C3H	Spleen	68	0			
(C3H X 101)F_1	(101 X C3H)F_1	Bone marrow	6	0			
	(C57BL/6 X DBA/2)F_1	Bone marrow	6	83	1	1	3
	C57BL/6 or DBA/2	Bone marrow	7	0			
	C57BL/6 or DBA/2	Spleen	14	0			
(C57BL X A)F_1	C57L or A	Bone marrow	7	0			
	C57L or A	Spleen	12	0			
(C57BL X C3H)F_1	C3H	Spleen	2	100		2	
	C57BL	Spleen	2	0			

*Radiation doses were 900–950 rads in animals receiving bone marrow and 400–900 rads in animals receiving spleen cells.

**Examined histologically between 7 and 30 days.

Table III summarizes the results of staining of fibrinoid lesions in the spleen with five staining procedures accepted as specific for fibrin.[6, 9-11] Comparison with fibrin from blood clots showed the lesions to contain numerous fibers that stained with the same reactions as fibrin. Positive reactions with aniline blue, fast green, and with the connective tissue stains are the same as described for collagen.[12] After treatment with PAS, the fibrinoid lesions reacted by coloring with a strong, positive red, thus indicating the presence of polysaccharide (Fig. 3). However, further treatment of parallel sections (fixed in formaldehyde and reinforced with dipyridinium chloride to bind acid and neutral mucopolysaccharides) with alcian blue at pH 2.5, toluidine blue, and Hale's colloidal iron failed to reveal significant amounts of any mucopolysaccharides (Fig. 4). A yellow (buff) color, persisting throughout the lesion when stained with colloidal iron, suggested the presence of protein, gycloprotein, or mucoprotein. Lesions stained with PTAH contained varying amounts of purple fibrous material surrounding the central arteriole (Figs. 5 and 6). Upon application of Foot's silver technique, a few fibers coated with silver spread throughout the lesion (Fig. 7). In view of Glynn and Loewi's report (1952) that fibrinoid is readily stained with silver, it is uncertain whether the black fibers seen in this lesion represent fibrinoid or reticulum. Sections of blood clot allowed to remain intraperitoneally in rats for 2 days showed an abundance of coarse and fine fibers of fibrin when the clot was stained with PTAH (Fig. 8). Amyloid was not found, although congo red, crystal violet, and thioflavin T (fluorescent) were applied to the lesions and compared with a positive amyloid control. Mallory's aniline blue-orange G stain gave a positive reaction for fibrin in fibrinoid lesions of all but 2 mice. Lesions in the 24- and 27-day animals stained blue with Mallory's, orange-yellow with PTAH, and green with the Picro-Gomori stain, suggesting the presence of collagen.

TABLE III. Summary of histochemical staining reactions.

Stain	Spleen Lesion	Glomerulosclerosis	Amyloid	Blood Clot
PTAH	Blue-purple	Orange	Yellow	Blue-purple
Weigert's fibrin	Blue-black	Few areas Blue-black	Purple	Blue-black
Picro-Gomori	Red	Red	Pink	Red
PAS plus orange G	Orange-red	Orange-red	Not stained	Orange-red
Mallory	Orange-red	Blue	Not stained	Orange-red

Comment

The significance of fibrinoid necrosis in the spectrum of histopathologic changes in secondary disease is not clear. Radiation alone and cell injection alone were not associated with fibrinoid necrosis (Table I). Two cases of mild fibrinoid necrosis were found in mice receiving 900 rads and isogeneic bone marrow cells. The remainder of the cases occurred in mice receiving parental spleen or bone marrow cells or allogeneic bone marrow cells after radiation. There were marked strain differences in the frequency of fibrinoid necrosis under similar experimental conditions (Table II). Differences were noted between the two parental strains used as cell donors for several strains of F_1 hybrids. The frequency of fibrinoid necrosis was not correlated with the severity of the secondary disease reaction nor with presence or absence of the donor presensitization used in some groups of mice. The presence of fibrinoid necrosis in secondary disease, previously reported by us,[1,2] was also reported by others under similar conditions.[13-16] Under the same conditions Micklem and associates[17-19] encountered eosinophilic material that gave positive amyloid stains. We and others mentioned have also noted hyalinization of the damaged areas in later stages.

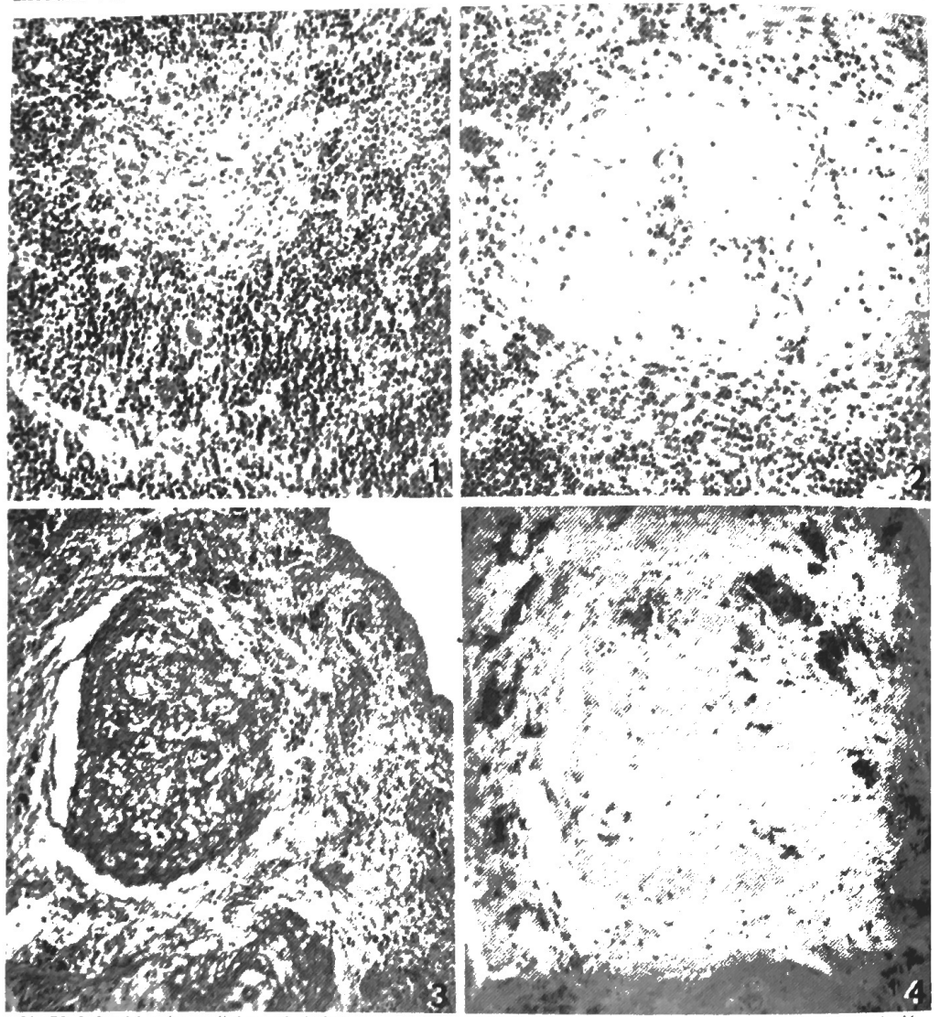

PLATE I. Special stains applied to splenic lesions. 1. Lesion in lymphatic nodule of spleen of 101 X C3H)F_1 mouse. The nodule is replaced by granulomatous reaction with some fibrinoid necrosis. 16 days after 950 rads + parental bone marrow cells (hematoxylin and eosin, X200). 2. Another lymphatic nodule from the mouse in 1, showing practically complete fibrinoid necrosis (Hematoxylin and eosin, X200). 3. Two lymphatic nodules, showing practically complete replacement of the nodule with fibrinoid material rich in polysaccharides. (RFM X AKR)F_1 mouse 22 days after 400 rads and parental spleen cells (PAS and hematoxylin, X150). 4. Absence of acid mucopolysaccharides in lesions. Dark-staining cellular areas surrounding the lesion indicate the presence of hemosiderin-laden macrophages. Same spleen as in 3 (Hale's colloidal iron, X150).

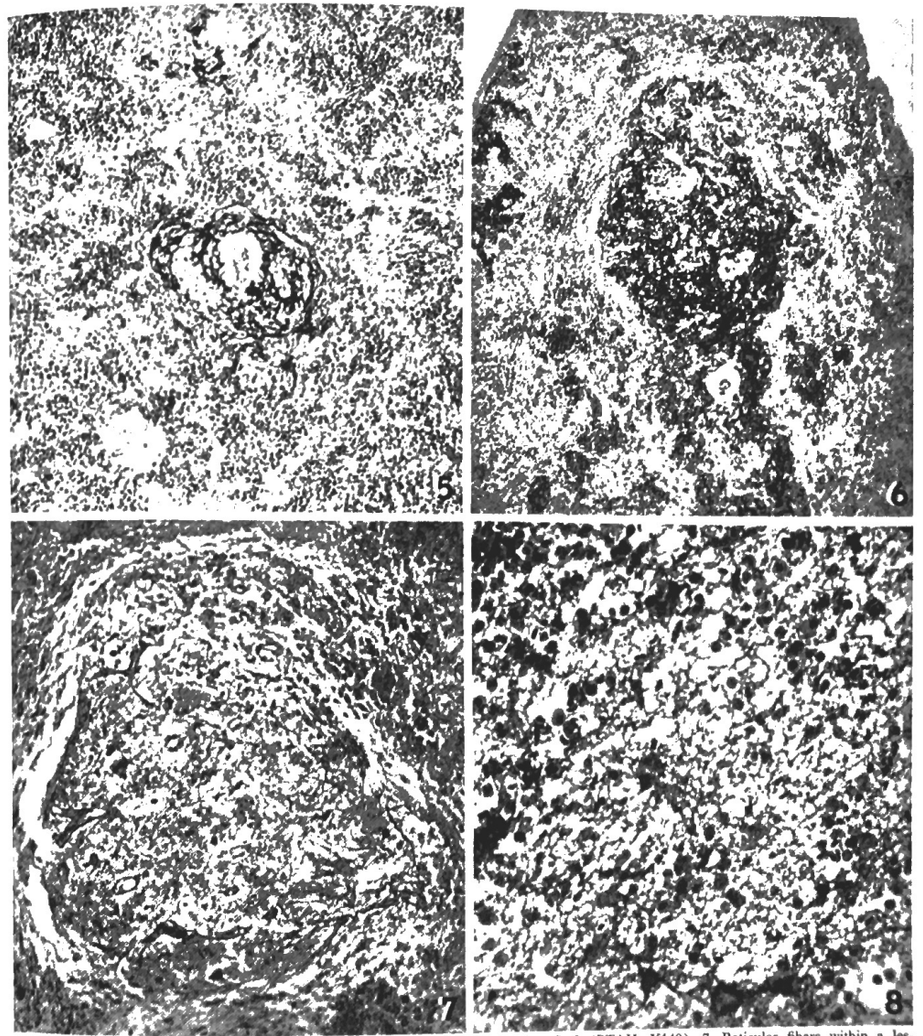

PLATE II. Special stains applied to splenic lesions. 5. Two lymphatic nodules, showing a positive staining reaction for fibrin in areas of fibrinoid necrosis surrounding the central arteriole. Same spleen as in Plate I, 1 (PTAH, X140). 6. More extensive fibrin deposit in fibrinoid necrosis. Same spleen as in Plate I, 3 (PTAH, X140). 7. Reticular fibers within a lesion. Same spleen as in Plate I, 3 (silver reticular stain, X190). 8. Two-day intraperitoneal blood clot, exhibiting many coarse and fine fibrin fibers. Sprague-Dawley rat 2 days after intraperitoneal injection of rat blood (PTAH, X400).

In a review Wagner[20] offered evidence for two possible origins of fibrinoid: alterations in connective tissue, specifically collagen fibers; and pathologically altered blood vessels. Supporters of nonhematogenous origin include Glynn and Loewi,[21] Strukov,[22] and Wagner.[20] Muirhead *et al.*[23] differentiated between "vascular" and "connective tissue" fibrinoid, suggesting that fibrinoid was derived from necrotic smooth muscle of the media of arterioles. Immunofluorescence studies strengthened the idea that fibrinoid originates in connective tissue diseases as well as from intravascular alterations somehow associated with coagulation mechanisms.[20] Also, numerous reports offer evidence that fibrinoid forms either as an insoluble derivative of fibrinogen[11] or during later stages in the degeneration of fibrinogen, resulting in the presence of fibrin within the lesion.[24-26]

Summary

Mice with secondary disease after irradiation and transplantation of parental spleen or allogeneic bone marrow deveolped fibrinoid necrosis in the splenic white pulp. Incidence and severity of this condition varied in different donor-recipient strain combinations. Radiation alone or injection of hemopoietic cells without radiation did not cause secondary disease or fibrinoid necrosis.

We applied to necrotic lesions in the spleen five stains commonly used to show fibrin. We observed that varying amounts of fibrin threads within the lesions reacted positively with these stains, thus revealing the presence of fibrin. The morphological and staining characteristics of the necrotic lesions were, however, identical with those generally described in fibrinoid necrosis. Stains for proteins, mucopolysaccharides, and amyloid were negative. Interpreting these findings as representative of one type of fibrinoid necrosis and not of fibrin alone, we agreed with the hypothesis that fibrinoid was formed from either plasma proteins or as a terminal step in the sequence of fibrinogen degeneration in areas of necrotic ground substance.

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