Virchows Archiv (1995) 426:61–67

ORIGINAL ARTICLE

Marsha L. Eigenbrodt · Joel S. Kneitz · Dwain L. Thiele
Edwin H. Eigenbrodt

Cellular blebbing in superficial colonic epithelial cells occurring with murine graft-versus-host disease

Received: 15 September 1994 / Accepted: 11 November 1994

Abstract Subnuclear blebbing of the superficial colonic epithelium, a rarely described light and electron microscopic change in graft-versus-host disease (GVHD), was studied in a murine model of GVHD. Severity of changes induced by transfer of various donor T cell subsets to irradiated, allogeneic recipients, and association with more severe alterations such as erosions and ulceration were evaluated. By light microscopy the basal region of the superficial enterocytes was greatly expanded by eosinophilic to amphophilic, flocculent, sometimes vacuolated material. By electron microscopy these changes were found to be organelle-poor, cytoplasm-filled protrusions from the basal surface of the epithelium. In this model, helper T cells (CD4+-enriched, CD8+-depleted T cells) transplanted after high dose irradiation were capable of causing the change suggesting cytokine responses may be involved in mediating the cellular injury seen histologically. Close association of blebbing and erosions suggest the blebbing may be the precursor to epithelial erosion or denudation seen in severe intestinal GVHD.

Key words Graft-versus-host disease · Cell blebs · Light microscopy · Electron microscopy

Introduction

Pathological findings in intestinal graft-versus-host disease (GVHD) are well described and include necrosis of individual crypt cells, crypt abscesses, crypt dilatation, crypt dropout, and a variable mononuclear inflammatory cellular infiltrate [10, 11, 26, 27, 32, 37, 38, 45, 47]. Lymphocytic infiltration and epithelial injury at the base of the crypts and point contact between lymphocytes and epithelial cells have been the main ultrastructural findings in several organs during GVHD [13, 14, 25, 26, 33, 50]. While diffuse intestinal ulceration or denudation has been reported in severe GVHD [26, 38, 47], other light microscopic changes in superficial colonic epithelial cells are rarely described. Epstein et al. [11] reported vacuolization of superficial epithelial cells as "lacunar change with cellular debris". Cytoplasmic blebbing, which is recognized as an ultrastructural change indicating early injury [2, 6, 12, 15, 19, 20, 22, 23], has been associated with GVHD of liver [28, 29] but has not been reported previously in intestinal GVHD. In our murine model of colonic GVHD there were many histological features similar to human GVHD including erosions and ulcerations. In addition, the colonic surface epithelium often developed subnuclear, cytoplasmic blebs which appeared on light microscopy as eosinophilic to amphophilic, amorphous, sometimes vacuolated material. The severity of the blebbing on light microscopy was compared in the various treatment groups to determine possible effector mechanisms. A possible association with erosions was also evaluated.

M.L. Eigenbrodt (✉) · E.H. Eigenbrodt
Department of Pathology and Laboratory Medicine,
University of Texas Medical School in Houston,
6431 Fannin Street, Houston, TX 77030, USA

J.S. Kneitz
Medical Student, MSIV,
University of Texas Medical School in Houston, 6431 Fannin,
Houston, TX 77030, USA

D.L. Thiele
Department of Internal Medicine,
University of Texas Southwestern Medical School,
Dallas, TX 75235, USA

Materials and methods

(C57BL/6JxDBA/2)F1 (B6D2F1), and DBA/2J (DBA/2) female mice were purchased from the Jackson Laboratory, Bar Harbor, Maine.

Anti-Thy1.2 (HO-13-4), anti-L3T4 (GK1.5), anti-Lyt2 (3.155) and anti-Lyt2.1 (116-13.1) were culture supernatants of ascitic fluid produced from hybridoma cells obtained from the American Type Culture Collection, Rockville, Md. [9, 18, 24, 34]. L-leucyl-L-leucine methyl ester (Leu-Leu-OMe) was synthesized from leucyl-leucine (Sigma, St. Louis, Mo.) as previously described [43] and was used to selectively deplete cytotoxic T lymphocyte (CTL) and natural killer (NK) cells as previously detailed [44].

Recipient mice between 8 and 16 weeks of age were maintained on acidified (pH 2), antibiotic (neomycin, 100 mg/l, and polymyxin B, 10 mg/l) water for 2–3 days before and 7 days after

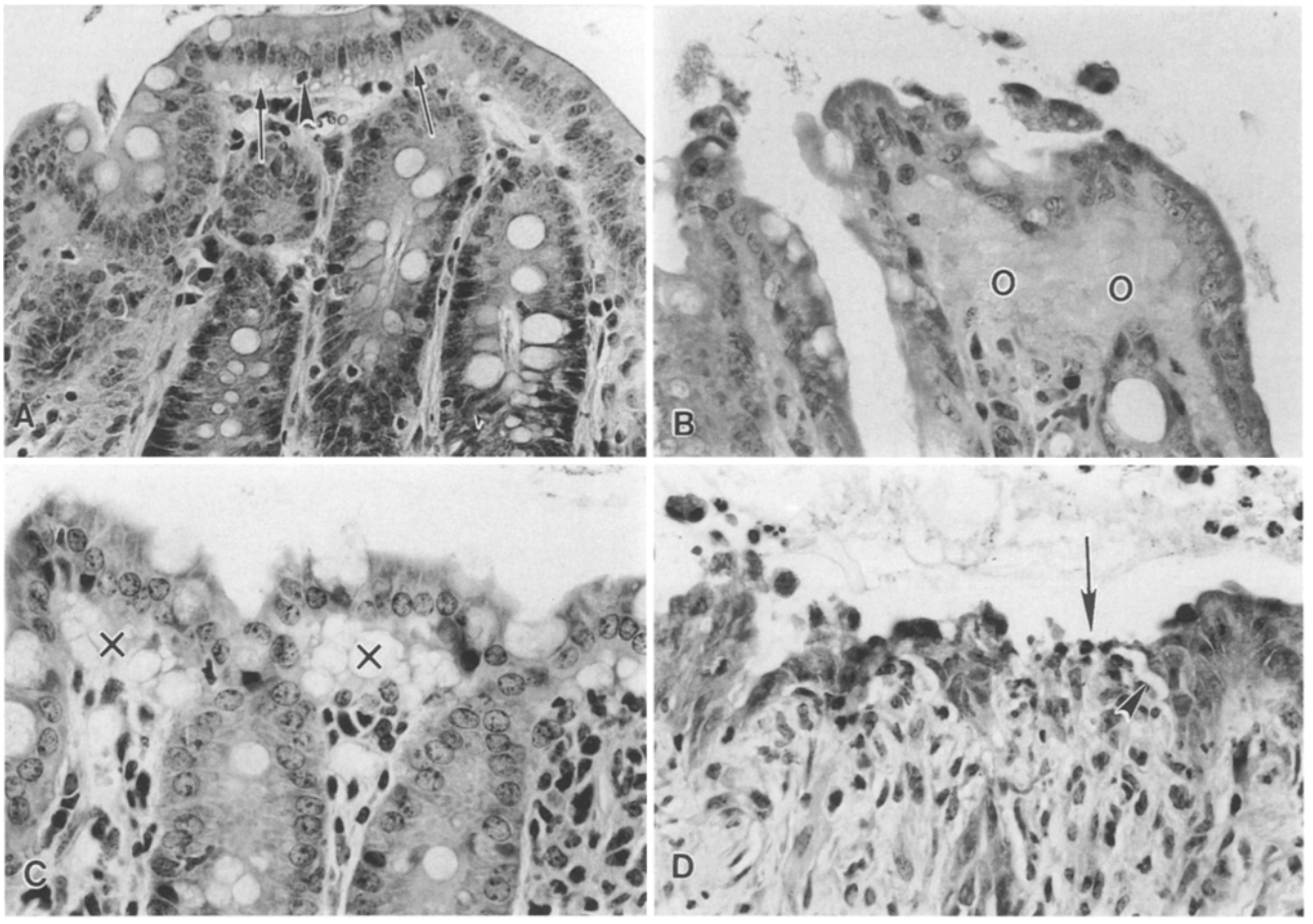

Fig. 1A–D Subnuclear blebs in the superficial colonic epithelium of mice with intestinal graft-versus-host disease (GVHD). Hematoxylin and eosin stain. **A** Early blebs (*arrows*) seen as small vacuoles of the basal cytoplasm of the surface colonic epithelium. Note intraepithelial lymphocyte (*arrowhead*). ×245. **B** Prominent blebbing with smudged appearance and loss of epithelial basal cell borders (*O*). ×375. **C** Prominent blebbing with vacuolated or bubbly appearance (*X*). ×375. **D** Early erosion (*arrow*) of the surface epithelium with blebbing (*arrowhead*) of epithelium adjacent to the erosion. Note attempt at regeneration with flattening of some epithelial cells and piling up of other cells near the erosion

Table 1 Light microscopic evaluation for blebs, erosions and epithelial regeneration

Donor Strain	Donor SpC treatment	B6D2F1 host irradiation	B6D2F1 recipients examined	B6D2F1 with blebbing[a]	Degree of blebbing[b]	B6D2F1 with erosions	B6D2F1 with epithelial regeneration
DBA/2	Anti-Thy 1.2+C′ — or — anti L3T4+C and anti Lyt2+C′	900	10	1	0.2±0.6	0	0
DBA/2	Nil or LLME	900	4	3	2.1±1.4	2	3
DBA/2	Anti L3T4+C′	900	7	5	1.4±1.3	1	4
DBA/2	Anti Lyt2+C′	900					
	+LLME		10	8	1.9±1.2	4	6
	--LLME		7	7	1.9± .9	6	7
	Total		17	15	1.9±1.1	10	13
DBA/2	Nil or LLME	600	5	0	0.0±0.0	0	0

[a] Blebbing indicates vacuoles or smudginess with loss of distinct cell borders in the basal regions of the surface epithelial cells

[b] Values represent the mean ± standard deviation of the mean for each group of the most severe degree of blebbing found in any of six colon sections in each animal (1 = least severe; 3 = most severe)

SpC = spleen cells

LLME = L-leucyl-L-leucine methyl ester (Leu-Leu-OMe)

transplantation. On the day of transplantation recipients (43 B6D2F1 mice) were irradiated (600 or 900 cGy) and 2–6 h later were injected via the lateral tail vein with donor cells in 0.5 ml Hanks' balanced salt solution. The donor cells consisted of 5×10^6 T-cell- and NK-cell-depleted DBA/2 bone marrow cells (anti-Thy1.2+C and Leu-Leu-OMe-treated) in addition to 5×10^6 DBA/2 spleen cells (SpC) depleted of T cells, enriched for helper T cells (+/– Leu-Leu-OMe), enriched for cytotoxic-suppressor T cells, depleted of NK and CTL (Leu-Leu-OMe), or receiving no treatment as previously described [10, 45]. Two B6D2F1 mice received no irradiation or transplantation and were used as normal controls.

From each mouse six sections of colon were paraffin-embedded and 4- to 5-μm sections were stained with haematoxylin and eosin and evaluated for GVHD as previously described [10]. Because of loss in tissue processing or slide breakage only 2–4 sections were available for evaluation in several mice. Each section was also evaluated for acute erosion or epithelial denudation and for re-epithelialization. Blebbing was graded from 1 to 3 as follows. The subnuclear cytoplasm of the superficial colonic epithelial cells had either definite vacuoles or loss of distinct cell borders in the basal region of the cells. Grade increased from 1 to 3 as the smudged or vacuolated area increased in thickness from over 1 nuclear diameter to over 3 nuclear diameters. Surface epithelial nuclei were used as an internal thickness gauge. All grading was performed blinded as to the donor cells and dose of irradiation the mouse had received.

Colons of four mice with blebbing on light microscopy were chosen and random cross-sections from colons fixed in Carson's formalin were embedded in Epon and thin sections were made. Thin sections were evaluated for the presence of blebbing and grids were prepared from the positive areas and studied by electron microscopy.

Results

Light microscopy

In normal, non-irradiated, non-transplanted mice the superficial colonic epithelial cells retain distinct cell borders, contain nuclei in a basal or mid-cellular location, and have no subnuclear cytoplasmic vacuoles, that is to say, no blebs are identified. These colons are graded less than 1. The light microscopic picture of blebbing varies from slight vacuolization of the basal portion of the superficial epithelial cells or smudging with loss of distinct cytoplasmic borders in the basal region (grade 1) to marked separation of the nuclei from the underlying lamina propria by pale pink to amphophilic, often vacuolated material simulating a markedly thickened basement membrane (grade 3) (Fig. 1A–C). Inflammatory cells are present in some areas of blebbing, but not in others.

By light microscopy, 23 of 28 mice treated with 900 cGy and some type of allogeneic T cells had blebbing, while only 1 of 10 mice receiving 900 cGy and no infusion of T cells (spleen cells treated with either anti Thy 1.2+C or anti-L3T4 and anti-Lyt2 +C) had blebbing. No mice receiving 600 cGy had blebbing. Of the mice receiving 900 cGy, 15 of 17 mice receiving helper enriched T cells with or without Leu-Leu-OMe, 3 of 4 mice receiving whole T cells, 5 of 7 mice receiving cytotoxic-suppressor enriched T cells, and only 1 of 10 mice receiving no T cells had blebbing (Table 1). Blebbing was found in 8 of 10 mice treated with 900 cGy and infused with helper enriched T cells even when the T cells were treated with Leu-Leu-OMe to remove all NK cells and to remove the ability to generate cytotoxic lymphocytes from both CD4+ and CD8+ lymphocytes.

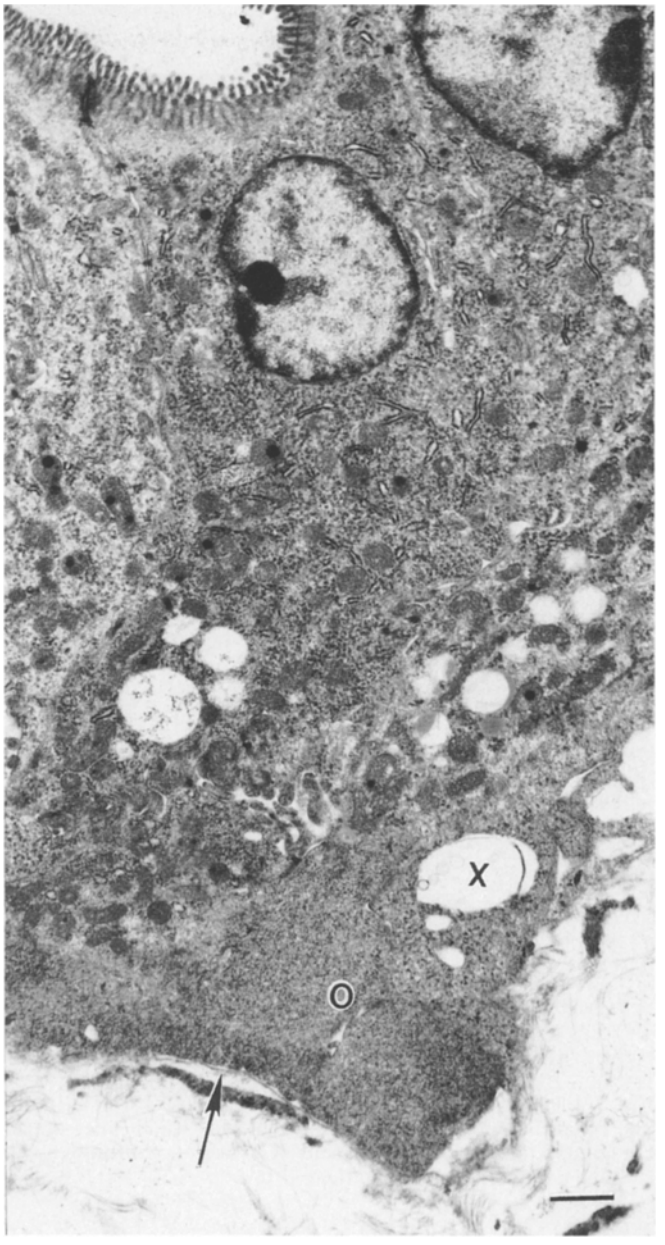

Fig. 2 Electron micrograph of early subnuclear bleb in superficial colonic epithelial cell seen as organelle-poor, granular area (*O*) with vacuoles (*X*). No membrane separates the early bleb from the cell of origin. Basal lamina (*arrow*) with foci of disorganization. *Bar* = 1 μm

Comparison of the mean grades for the degree of blebbing in each group is shown in Table 1. There was no statistically significant difference in the degree of blebbing found in mice receiving 900 cGy and whole T cells compared to any other group receiving 900 cGy and any subtype of allogeneic T cell using the unpaired Student *t*-test (two-tailed analysis). The degree of blebbing found in the combined groups of animals receiving

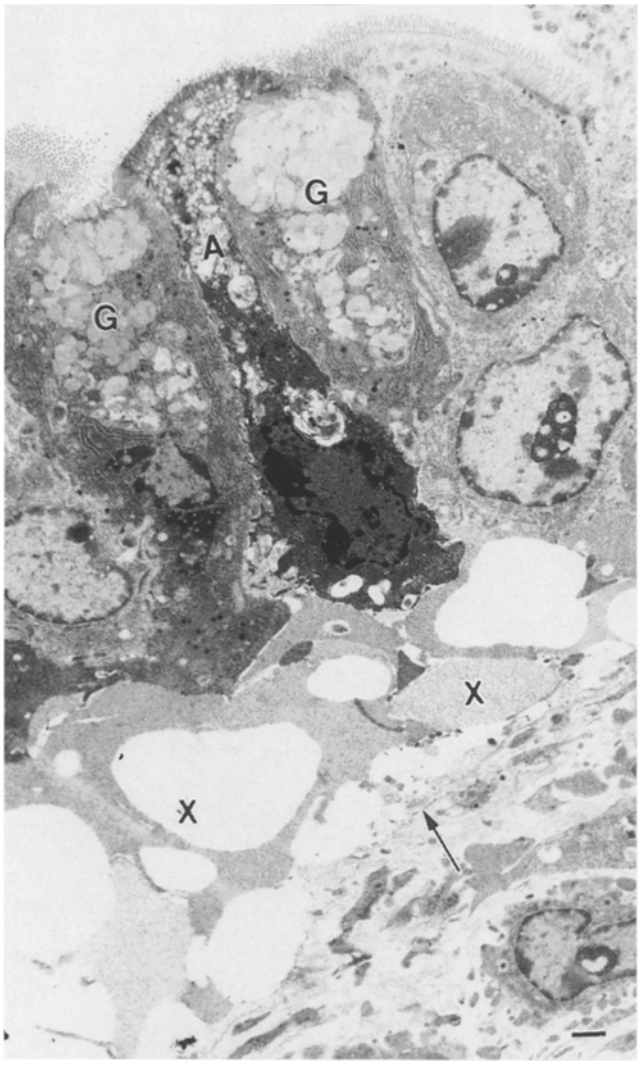

Fig. 3 Electron micrograph of prominent blebs (*X*) seen as multiple, organelle-poor, granular or vacuolated, partially membrane-bound structures. Note goblet cells (*G*), necrotic absorptive cell (*A*), and basal lamina (*arrow*). *Bar* = 1 μm

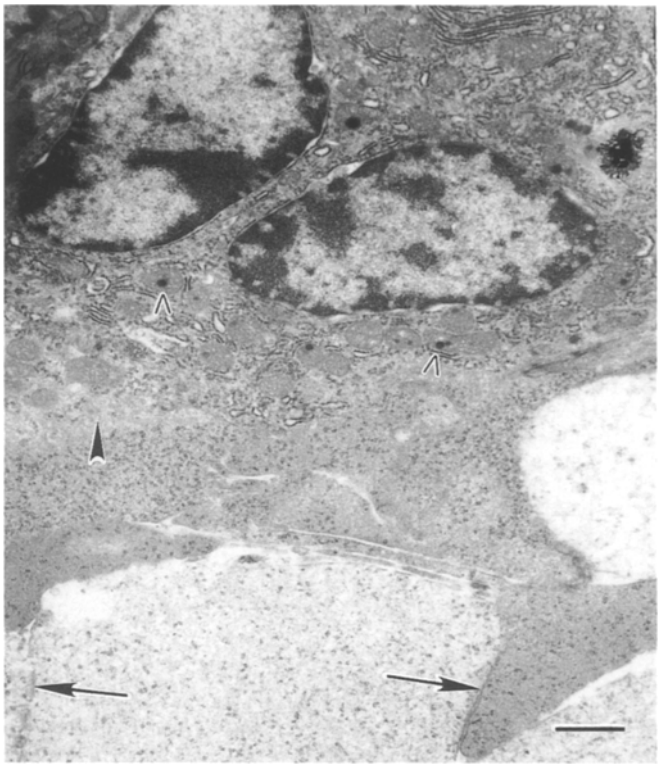

Fig. 4 Electron micrograph of the junction of blebbing with overlying enterocyte cytoplasm. Note membranes between blebs (*arrows*) and only partial separation of the blebs from the overlying cytoplasm. Cytoskeletal filaments (*arrowhead*) are sometimes interposed between the blebs and the overlying organelle-containing cytoplasm. Amorphous densities are present in mitochondria (>). *Bar* = 1 μm

900 cGy and any type of allogeneic T cell was significantly greater than in animals receiving whole spleen cells and 600 cGy ($P<0.001$, non-paired, two-tailed Student *t*-test) or mice receiving 900 cGy and no T cells ($P<0.001$, non-paired, two-tailed Student *t*-test). The degree of blebbing for each group of mice treated with 900 cGy and infused with any type of allogeneic T-cell subset was significantly greater than the severity of blebbing seen in either group receiving 600 cGy and T cells or 900 cGy and no T cells ($P = 0.016$ to 0.0005 for non-paired, one-tailed Student *t*-test). There was no significant difference in the degree of blebbing seen in mice receiving 900 cGy and no T cells compared to mice receiving 600 cGy and whole T cells.

Epithelial denudation or erosions (Fig. 1D) were found only in mice in which there was blebbing, but blebbing was not always accompanied by erosion. The epithelium adjacent to most erosions appeared regenerative with clustering of cells containing large, active nuclei and cytoplasm lacking a columnar configuration. Sometimes epithelium adjacent to erosions had basilar vacuolization or a smudgy appearance suggestive of blebbing (Fig. 1D).

Fourteen of 28 mice treated with 900 cGy and some type of allogeneic T cells had erosions, ulcers or epithelial denudation. None of these changes were found in mice receiving 900 cGy and no T cells or in mice receiving 600 cGy and T cells.

Electron microscopy

On electron microscopy of intact, non-eroded but blebbed surface epithelium, the expanded subnuclear region is identified as organelle-poor, cytoplasm-filled protrusions from the basal epithelial surface. The earliest findings by electron microscopy are basally located vacuoles and/or organelle-depleted, granular, expanded areas in the basal region of the surface epithelium (Fig. 2). Ribosomes are present, but there is a marked lack of other organelles compared to the remainder of the enterocyte cytoplasm. More prominent blebbing shows numerous rounded, partially membrane-bound blebs with contents as described for the early blebs (Fig. 3). The most

superficial blebs are not completely separated from the adjacent cell by membranes. In some areas there is a slight increase in cytoskeletal filaments between the organelle-containing cytoplasm and the organelle-depleted blebs (Fig. 4). Many of the cells with blebs have other electron microscopic features of injury including amorphous densities in mitochondria, clustering of mitochondria, large nuclei with loss of matrix density and chromatin material and cytoplasmic vacuoles and dilated endoplasmic reticulum. Only in severely injured cells are the surface microvilli distorted. In some areas of blebbing there are inflammatory cells, either neutrophils, eosinophils, macrophages, or lymphocytes.

Discussion

In our murine model of intestinal GVHD, which had many histological similarities to human intestinal GVHD [10, 45], we recognized a histological feature that was found, on electron microscopy, to consist of cytoplasmic protrusions of the basal region of the superficial colonic epithelial cells. On light microscopy the basal region of the superficial colonic epithelial cells had changes that varied from mild vacuolization to a broad, bubbly, pale pink, amorphous area which on light microscopy appeared to be thickened basement membrane. While initially dismissed as non-specific, further study revealed a statistically significant association of blebbing with GVHD. No blebs were present in mice receiving low-dose irradiation and bone marrow with T cells, and only one of ten mice receiving high-dose irradiation and bone marrow without T cells developed blebbing. There was a much higher frequency and a statistically significant greater degree of blebbing in the groups of animals which developed GVHD (that is, in mice receiving high-dose irradiation and allogeneic spleen cells, Table 1). Blebbing thus does appear to be a histological feature, but not a specific one, associated with intestinal GVHD.

Vacuolization by light microscopy and blebbing recognized ultrastructurally has been associated with other sites of GVHD. While not a light microscopic feature commonly mentioned in intestinal GVHD, vacuolization of basal epithelial cells (vacuolar degeneration) with occasional bullae were identified in skin, tongue, esophagus, and urinary bladder in animals and humans and were attributed to the affects of GVHD [4, 25, 36, 37, 40, 42, 46, 49–51]. These changes differ histologically from blebbing in this study in that the entire cytoplasm (rather than just the basal or subnuclear region) of the basal cells are vacuolated and there is abundant intercellular edema with a lymphocytic infiltrate. Reports of the ultrastructural changes of skin and gut GVHD have emphasized the point contact of lymphocytes with epithelial cells resulting in epithelial injury. In skin the basal cell layer is most severely involved and in the gut the crypts are the primary area of attack [14, 16, 33, 50]. Changes of cytoplasmic blebs have been reported in the ultrastructural studies of small bile ducts; however, in contrast to those in the colon of our mice, the bile duct blebs were apically, not basally, located [28, 29].

In a study by Epstein et al. [11] of the accuracy of rectal biopsy in human intestinal GVHD, a histological change similar to the subnuclear vacuolization observed in our mice is seen in a photomicrograph of rectal epithelium and is described as "lacunar change with epithelial debris" in their Fig. 1E. The adjacent cells have basilar vacuolization similar to that seen in some of our mice. One reason that this finding has received little attention in human GVHD is that hypertonic enemas and ischemia of any cause have been reported to produce similar light microscopic changes [17, 48]. However, the significance of blebbing as a histological feature in GVHD should not be overlooked. Cytoplasmic blebbing is a well-recognized early ultrastructural alteration of cell injury in a variety of different cells from a number of different injurious agents such as toxins, viruses, hypoxia, irradiation, and disruption of extracellular matrix [1, 2, 12, 15, 19, 20, 22, 23, 41, 48]. Also, in our mice, blebbing is associated with more severe changes of erosions or ulcerations. These facts indicate the subnuclear epithelial blebs are significant changes of intestinal injury.

The specific mechanism of blebbing in this model of GVHD is not clear. Irradiation is a well-recognized cause of intestinal injury. Early ultrastructural changes in the small intestine include decrease in epithelial cell mitotic rate, mitochondrial damage and/or aggregation, proliferation and dilation of the smooth endoplasmic reticulum, appearance of numerous primary lysosomes, appearance of immature crypt-type cells on small intestinal villi, crypt cell death, separation of the epithelial cells from the lamina propria (leaving extracellular space, but no cell blebs on electron microscopy), appearance of multinucleated giant cells, protrusion of the basal region of epithelial cells of the intestinal villus through the basement membrane into the underlying lamina propria, endothelial cell damage, occasional focal erosions and eventual villus shortening [7, 21]. By light microscopy early changes in the small intestine include pyknosis and karyorrhexis of proliferating cells with death and sloughing of cells leading to erosions, denudation and eventual villous atrophy, inflammatory cells in the lamina propria and endothelial swelling. Light microscopic changes in the colon are similar with additional features of loss of nuclear polarity, enlarged nuclei, loss of mucin production or swelling of goblet cells, appearance of numerous crypt abscesses, edema, and accumulation of inflammatory cells such as neutrophils and eosinophils within the stroma [3, 5, 35]. While some of the features of injury in this model of GVHD are similar to ultrastructural changes found in irradiation, irradiation alone did not cause all of the changes. Resolution of the acute effects of irradiation has been reported by day 15 and day 20 post-irradiation by De Vries and Vos [8] in radiation chimeras and by McDonald et al. [27] in rectal biopsies of transplant patients, respectively. Since the mice in this study were evaluated at around day 25, it is unlikely that the histological changes seen were due to irradiation alone. In

fact, in this study only one mouse out of the ten which received 900 cGy and T-cell-depleted bone marrow cells developed blebs, indicating that factors other than irradiation were involved. The occurrence of significantly more blebbing in irradiated mice transplanted with bone marrow containing T cells suggests GVHD is an underlying cause. CD8+ (Lyt2+) T cells, when stimulated with class I MHC alloantigens, can produce cytokines and are not exclusively cytotoxic [30, 44]. The presence of blebbing in irradiated mice transplanted with bone marrow enriched for CD4+ (L3T4+) T cells and depleted of cytotoxic T cells suggests that cytokines secreted by the helper T cells may be involved in the injury causing the blebs. Indeed, the induction of cytokine secretion by lymphocytes has been postulated as the mechanism by which infections make bone marrow transplant recipients targets for GVHD [39]. In a study of the immunopathology of upper gastrointestinal acute GVHD, findings suggested that inflammatory cell activation and resultant secretion of cytokines might directly damage the mucosa or act by recruitment of additional cytotoxic lymphocytes [31]. In our study indirect injury could result from stimulation of macrophage cytotoxicity or activation of NK cells or cytotoxic lymphocytes by interferon gamma or interleukin 2 in mice transplanted with whole spleen cells or cytotoxic lymphocytes. Blebbing in the subset of mice transplanted with spleen cells enriched for helper T cells and treated with Leu-Leu-OMe suggests a more direct mechanism of injury by cytokines such as tumor necrosis factor or lymphotoxin since these mice lack donor cytotoxic lymphocytes and NK cells. However, indirect injury from stimulation of macrophage cytotoxicity cannot be excluded in this subset of mice. High-dose irradiation was additive or synergistic in the development of blebbing since T cells with only 600 cGy did not cause colonic GVHD or blebs in this strain of mice. Additional work is needed to further delineate the mechanism of injury.

In conclusion cytoplasmic or plasma membrane blebbing is a well-recognized morphological alteration of cell injury in a number of tissues from a number of different injurious agents but has not been previously emphasized in intestinal GVHD. The occurrence of blebs significantly more frequently in mice with GVHD than the mice without GVHD and the association with erosions indicates the change is a significant one. In our model, cytoplasmic blebbing of the basal region of superficial colonic epithelial cells was found in mice treated with high dose irradiation and helper T cells, suggesting a role for cytokines in the production of blebs. However, the occurrence of blebbing in these mice may be a reflection of the severity of injury occurring in the intestine rather than an indication of a specific mechanism.

References

1. Amerongen HM, Mack JA, Wilson JM, Neutra MR (1989) Membrane domains of intestinal epithelial cells: distribution of Na^+, K^+-ATPase and the membrane skeleton in adult rat intestine during fetal development and after epithelial isolation. J Cell Biol 109:2129–2138
2. Berger ML, Reynolds RC, Combes B (1987) Carbon tetrachloride-induced morphologic alterations in isolated rat hepatocytes. Exp Mol Pathol 46:245–257
3. Berthrong M, Fajaardo LF (1981) Radiation injury in surgical pathology. II. Alimentary tract. Dig Dis Sci 34:390–399
4. Beschorner WE, Tutschka PJ, Santos GW (1982) Sequential morphology of graft- versus-host disease in the rat radiation chimera. Clin Immunol Immunopathol 22:203–224
5. Buell MG, Harding RK (1989) Proinflammatory effects of local abdominal irradiation on rat gastrointestinal tract. Dig Dis Sci 34:390–399
6. Buja LM, Eigenbrodt ML, Eigenbrodt EH (1993) Apoptosis and necrosis: basic types and mechanisms of cell death. Arch Pathol Lab Med 117:1208–1214
7. Carr KE, Toner PG, McLay ALC, Hamlet R (1981) The ultrastructure of some gastrointestinal lesions in experimental animals and man. Scand J Gastroenterol [Suppl] 70:107–128
8. De Vries MJ, Vos O (1959) Delayed mortality of radiation chimeras: a pathological and hematological study. J Natl Cancer Inst 23:1403–1439
9. Dialynas DP, Wilde DB, Marrack P, Pierres A, Wall KA, Havran W, Otten G, Loken MR, Pierres M, Kappler J, Fitch FW (1983) Characterization of the murine antigenic determinant, designated L3T4a by functional T cell clones appears to correlate primarily with class II MHC antigen-reactivity. Immunol Rev 74:29–56
10. Eigenbrodt ML, Eigenbrodt EH, Thiele DL (1990) Histologic similarity of murine colonic graft-versus-host disease (GVHD) to human colonic GVHD and inflammatory bowel disease. Am J Pathol 137:1065–1076
11. Epstein RJ, McDonald GB, Sale GE, Shulman HM, Thomas ED (1980) The diagnostic accuracy of the rectal biopsy in acute graft-versus-host disease: a prospective study of thirteen patients. Gastroenterology 78:764–771
12. Gabai VL, Kabakov AE, Mosin AF (1992) Association of blebbing with assembly of cytoskeletal proteins in ATP-depleted EL-4 ascites tumor cells. Tissue Cell 24:171–177
13. Gallucci BB, Shulman HM, Sale GE, Lerner KG, Caldwell LE, Thomas BA, Thomas ED (1979) The ultrastructure of human epidermis in chronic graft-versus-host disease. Am J Pathol 95:643–653
14. Gallucci BB, Epstein R, Sale GE, Shulman HM, McDonald GB, Thomas ED (1982) The fine structure of human rectal epithelium in acute graft-versus-host disease. Am J Surg Pathol 6:293–305
15. Gores GJ, Herman B, Lemasters JJ (1990) Plasma membrane bleb formation and rupture: a common feature of hepatocellular injury. Hepatology 11:690–698
16. Gullen FJ, Ferrara J, Hancock WW, Messadi D, Fonferko E, Burakoff SJ, Murphy GF (1986) Acute cutaneous graft-versus-host disease to minor histocompatibility antigens in a murine mode. Lab Invest 55:35–42
17. Haggit RC (1983) The differential diagnosis of idiopathic inflammatory bowel disease. In: Norris HT (ed) Pathology of the colon, small intestine and anus. Churchill Livingstone, New York, pp 21–59
18. Hammerling G, Hammerling U, Kearney J (1981) Monoclonal antibodies and T-cell hybridomas. Elsevier/North-Holland, Amsterdam
19. Herman B, Nieminen A-L, Gores GJ, Lemasters JJ (1988) Irreversible injury in anoxic hepatocytes precipitated by an abrupt increase in plasma membrane permeability. FASEB J 2:146–151
20. Lemasters JJ, DiGuiseppi J, Nieminen A, Herman B (1987) Blebbing, free Ca^{2+} and mitochondrial membrane potential preceding cell death in hepatocytes. Nature 325:78–81
21. Lieb RJ, McDonald TF, McKenney JR (1977) Fine-structural effects of 1200-R abdominal × irradiation on rat intestinal epithelium. Radiat Res 70:575–584
22. Malorni W, Fiorentini C, Paradisi S, Giuliano M, Mastrantonio P, Donelli G (1990) Surface blebbing and cytoskeletal changes induced in vitro by toxin B from clostridium difficile: an immunochemical and ultrastructural study. Exp Mol Pathol 52:340-356

23. Malorni W, Iosi F, Mirabelli F, Bellomo G (1991) Cytoskeleton as a target in menadione-induced oxidative stress in cultured mammalian cells: alterations underlying surface bleb formation. Chem Biol Interact 80:217–236
24. Marshak-Rothstein A, Fink P, Gridley T, Raulet DH, Bevan MJ, Gefter ML (1979) Properties and applications of monoclonal antibodies directed against determinants of the Thy-1 locus. J Immunol 122:2491–2497
25. Martin PJ (1987) Graft vs host disease. In: Dixon FJ (ed) Advances in immunology. Harcourt Brace Javanowich, Orlando, pp 395–439
26. McDonald GB, Sale GE (1984) The human gastrointestinal tract after allogenic marrow transplantation. In: Sale GE, Shulman HM (eds) The pathology of bone marrow transplantation. Masson, New York, pp 77–103
27. McDonald GB, Shulman HM, Sullivan KM, Spencer GD (1986) Intestinal and hepatic complications of human bone marrow transplantation, part I. Gastroenterology 90:460–477
28. Nonomura A, Kono N, Yoshidak, Nakanuma Y, Ohta G (1988) Histopathologic changes of bile duct in experimental graft-versus-host disease across minor histocompatibility barriers. Electron microscopic observations. Liver 8:32–41
29. Nonomura A, Kono N, Mizukzmi V, Matsubaara F (1991) Histopathological changes in the liver in experimental graft-versus-host disease across minor histocompatibility barriers. A light and electron microscopic study of the periportal changes. Liver 11:278–286
30. Rosenberg AS, Mizuochi T, Singer A (1988) Evidence for involvement of dual-function T cells in rejection of MHC class I disparate skin grafts. J Exp Med 168:33–45
31. Roy J, Platt JL, Weisdorf JD (1993) The immunopathology of upper gastrointesinal acute graft-versus-host disease. Transplantation 55:572–578
32. Sale GE, McDonald GB, Shulman HM, Thomas ED (1979) Gastrointestinal graft-versus-host disease in man. Am J Surg Pathol 3:291–299
33. Sale GE, Galluci BB, Schubert MM, Sullivan KM, Thomas ED (1987) Direct ultrastructural evidence of target directed polarization by cytotoxic lymphocytes in lesions of human graft-versus-host disease. Arch Pathol Lab Med 111:333- 336
34. Sarmiento M, Glasebrook AL, Fitch AFW (1980) IgG or IgM monoclonal antibodies reactive with different determinants of the molecular complex bearing Lyt2 antigen block T cell-mediated cytolysis in the absence of complement. J Immunol 125:2665–2672
35. Sher ME, Bauer J (1990) Radiation induced enteropathy. Am J Gastroenterol 85:121- 128
36. Sloane JP, Norton J (1993) The pathology of bone marrow transplantation. Histopathology 22:201–209
37. Snover DC (1989) Biopsy interpretation in bone marrow transplantation. In: Rosen PP, Fechner RE (eds) Pathology annual, vol 24. Appleton & Lange, Norwalk, Conn, pp 63–101
38. Spencer GD, Shulman HM, Myerson D, Thomas D, McDonald GB (1986) Diffuse intestinal ulceration after marrow transplantation: a clinicopathologic study of 13 patients. Hum Pathol 17:621–633
39. Storb R (1991) Pathogenesis and recent therapeutic approaches to graft-versus-host disease. J Pediatr 118:S10–S13
40. Storb R, Thomas ED (1985) Graft-versus-host disease in dog and man: the Seattle experience. In: Moller G (ed) Immunological reviews. Munksgaard, Copenhagen, pp 215–238
41. Sugrue SP, Hay ED (1981) Response of basal epithelial cell surface and cytoskeleton to solubilized extracellular matrix molecules. J Cell Biol 91:45–54
42. Sullivan KM (1983) Graft-versus-host disease. In: Blume KG (ed) Clinical bone marrow transplantation. Churchill Livingstone, New York, pp 91–129
43. Thiele DL, Lipsky PE (1985) Regulation of cellular function by products of lysosomal enzyme activity: elimination of human natural killer cells by a dipeptide methyl ester by monocytes or polymorphonuclear leukocytes. Proc Natl Acad Sci USA 82:2468–2472
44. Thiele DL, Charley MR, Calomeni JA, Lipsky PE (1987) Lethal graft-versus-host disease across major histocompatibility barriers: requirement for leucyl-leucine methyl ester sensitive cytotoxic T cells. J Immunol 138:51–57
45. Thiele DL, Eigenbrodt ML, Bryde SE, Eigenbrodt EH, Lipsky PE (1989) Intestinal graft-versus-host disease is inhibited by donor T cells distinct from classic cytotoxic T cells distinct from classic cytotoxic T lymphocytes. J Clin Invest 84: 1947–1956
46. Thomas ED, Storb R, Clift RA, Fefer A, Johnson LF, Neiman PF, Lerner KG, Glucksberg H, Buckner CD (1975) Bone marrow transplantation (second of two parts). N Engl J Med 292:895–902
47. Thorning D, Howard JD (1986) Epithelial denudement in the gastrointestinal tracts of two bone marrow transplant recipients. Hum Pathol 17:560–566
48. Wagner R, Gabbert H (1983) Morphology and chronology of ischemic mucosal changes in the small intestine: a light and electron microscopic investigation. Klin Wochenschr 61:593–599
49. Wick MR, Moore SB, Gastineau DA, Haagland HC (1983) Immunologic, clinical and pathologic aspects of human graft-versus-host disease. Mayo Clin Proc 58:603- 612
50. Woodruff JM, Wilhelmina I, Butcher BS, Hellerstein LJ (1972) Early secondary disease in the Rhesus monkey. Electron microscopic changes in mucous membranes and external epithelia as demonstrated in the tongue and lip. Lab Invest 27:85–98
51. Woodruff JM, Hansen JA, Good RA, Santos GW, Slavin RE (1976) The pathology of graft-versus-host reaction (GVHR) in adults receiving boen marrow transplants. Transplant Proc 8:675–684