

Idiopathic capillary leak syndrome: Evidence of CD8-positive lymphocytes surrounding damaged endothelial cells

Marco Cicardi, MD,^a Emilio Berti, MD,^b Valentina Caputo, MD,^c
Ferdinando Radice, MD,^c Marco Gardinali, MD,^a and Angelo Agostoni, MD^a
Milan, Italy

Idiopathic capillary leak syndrome (ICLS) is a lethal disease characterized by recurrent fluid extravasation leading to hypovolemic shock underlain by marked hemoconcentration (hematocrit as high as 80%).¹ Only 30 patients with ICLS have been reported in the literature, and the pathogenesis remains unknown. Extravasation of intravascular fluid, with similar clinical symptoms, is also a well-known complication of systemic administration of recombinant (r)IL-2.² In this last condition, direct in vivo interaction between IL-2-activated cells (probably LAK cells) and endothelium results in cytotoxicity to endothelial cells.³

We previously provided evidence that cytokines participate in the pathogenesis of ICLS by demonstrating the appearance of IL-2 receptor-positive cells during attacks in a patient with this disease.⁴ Now we have studied the pathology of the same patient, who died during an attack, and have found that in the skin there were perivascular infiltrates of CD8 lymphocytes surrounding damaged endothelial cells, a picture similar to that of rIL-2-dependent capillary leak syndrome (CLS).

METHODS

The patient was a 56-year-old woman. Episodes of hemoconcentration had started 6 years earlier and continued with monthly recurrence for 2 years, when she was given 240 mg/day verapamil (to prevent any possible damage induced by Ca⁺⁺ influx). During the next 4 years she remained fairly stable, experiencing only initial symptoms that spontaneously subsided without hospitalization. In May 1993, she was admitted to our ward with clear signs of hypovolemic shock; the symptoms had started 24 hours earlier. The hematocrit value was 72%. The administration of fluids, plasma expanders, steroids, and dopa-

Abbreviations used

APAAP:	Alkaline phosphatase-antialkaline phosphatase
CLS:	Capillary leak syndrome
ICLS:	Idiopathic capillary leak syndrome

mine did not improve her clinical condition or laboratory parameters; and she died 16 hours later.

Three hours after death, skin samples were removed from the left shoulder and the abdomen, and a piece of muscle was removed from the deltoid. These specimens were fixed with standard procedures for light and electron microscopy. Immunohistochemical studies were performed on paraffin-embedded tissue sections by using rabbit polyclonal antibodies anti-CD3 (Dako, Denmark) and anti-S100 (Dako) and the following monoclonal antibodies: anti-CD45 RA-4KB5 (Dako), anti-CD20-L26 (Dako), anti-CD1a-010 (Immunotech), anti-CD45 RO (Dako-UCHL1), anti-CD 8^s (a gift from Prof. D. J. Mason), anti-CD30-Ber H2 (Dako), anti-CD68-KP1 (Dako), and PCNA (PC-10, Dako). To use the anti-CD8 antibodies, tissue sections were exposed to microwave radiation (95°C) for 10 minutes (two 5-minute periods). These antibodies were revealed with swine anti-rabbit (Dako) (for polyclonal antibodies) or rabbit anti-mouse (Dako) (for monoclonal antibodies) and the alkaline phosphatase-antialkaline phosphatase (APAAP) method (Cordel).

RESULTS AND DISCUSSION

As in previous reports,^{1, 6, 7} necroscopic examination failed to reveal any pathologic changes in our patient's internal organs. In particular, there were no signs of edema, and weights tended to be in the lower range of normal. These necroscopic data and the absence of clinical or laboratory evidence of organ failure during attacks suggest that plasma extravasation is not a systemic event in ICLS. On the contrary, the skin was diffusely edematous, and all specimens collected early after death had pronounced perivascular edema and accumulation of lymphomononuclear cells in the papillary and reticular layers of the dermis (Fig. 1, A). Immunophenotyping of these cells with monoclonal antibodies highlighted a population of primed T lymphocytes belonging to the CD3, CD45RO subset (Fig. 1, C

From ^athe Institute of Internal Medicine, ^bthe Institute of Dermatological Sciences, and ^cthe Institute of Pathologic Anatomy, University of Milan and Istituto di Ricovero e Cura a Carattere Scientifico, Ospedale Maggiore, Milan.

Received for publication Oct. 30, 1995; revised July 1, 1996; accepted for publication July 29, 1996.

Reprint requests: Angelo Agostoni, MD, Istituto di Medicina Interna, Via Pace 9, 20122 Milano, Italy.

J Allergy Clin Immunol 1997;99:417-9.

Copyright © 1997 by Mosby-Year Book, Inc.

0091-6749/97 \$5.00 + 0 1/177347

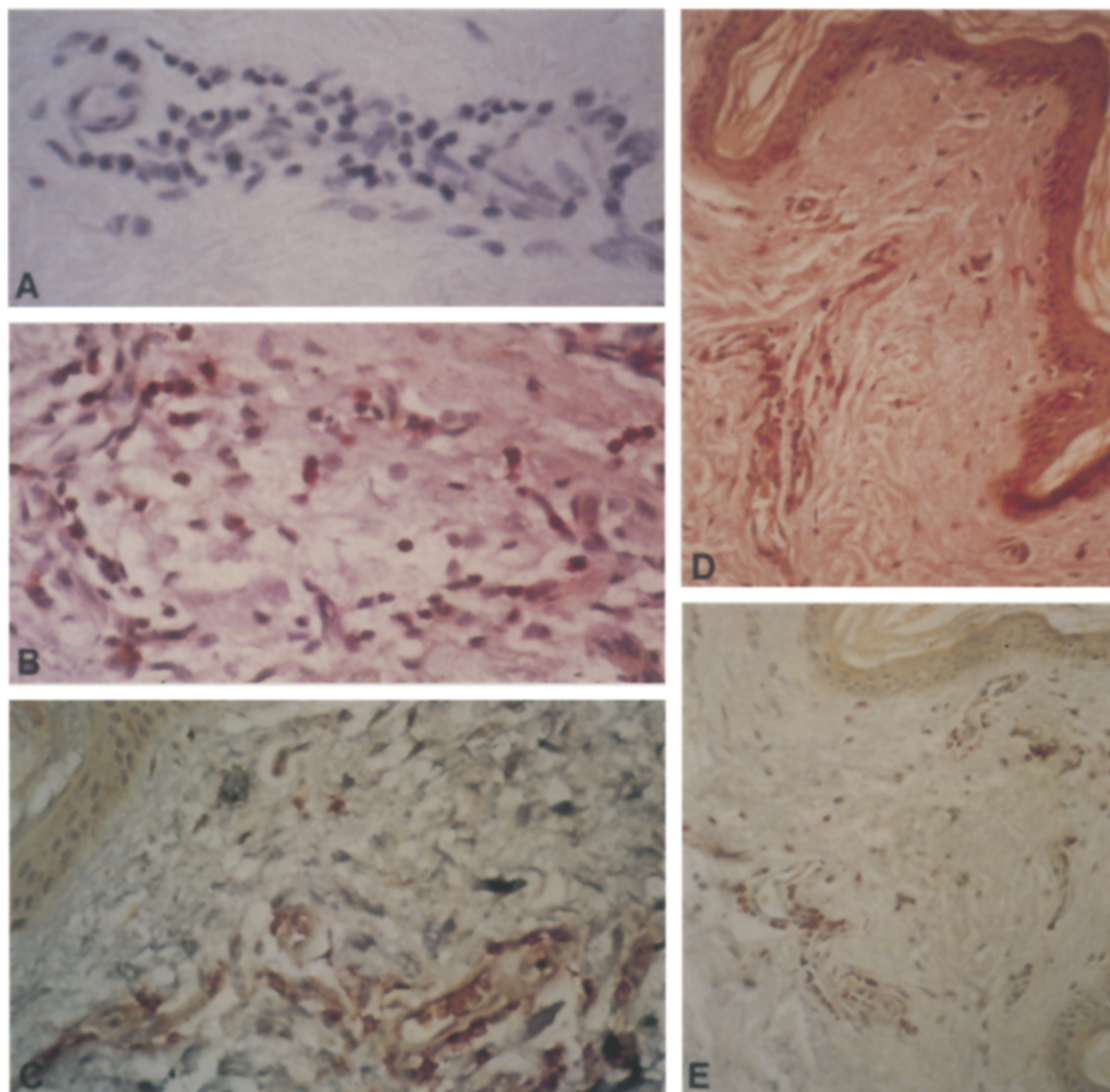


FIG. 1. **A**, Hematoxylin-eosin-stained skin section showing a mononuclear lymphoid infiltrate around a vessel in superficial dermis. **B**, Immunohistochemistry (anti-CD8-APAAP staining) of the same area showing several CD8-positive lymphocytes. **C**, Immunohistochemistry (anti-CD3-APAAP staining) showing T lymphocytes around vessels. **D**, Immunohistochemistry (anti-S-100-APAAP staining) showing many positive cells in epidermis and superficial dermis. Note the presence of some intravascular positive cells. **E**, Immunohistochemistry (anti-CD45RO-APAAP staining) showing that most lymphocytes are primed T cells.

and *E*). About 50% of these T lymphocytes expressed the CD8 phenotype (Fig. 1, *B*). This finding was obtained by counting the CD3/CD8 ratio in 10 serial sections. In the upper dermis there were a number of CD1a/S100 dendritic cells, which were also present within the intravascular space (Fig. 1, *D*). Ultrastructural analysis did not reveal any intracytoplasmic Birbeck granules in the CD1a/S100-positive cell in close proximity to the lymphoid population. Endothelial cells were damaged by intracellular edema that appeared in the form of vacuoles not surrounded by membranes that often destroyed the cytoplasmic structure. This lesion was particularly evident in those vessels surrounded by a mononuclear lymphoid infiltrate, which showed cyto-

toxic granules, but direct contact could not be demonstrated. The structure of the resident cell populations of the dermis was maintained. The intercellular junctions were not dilated, and the basement membrane was preserved. No infiltrates or other pathologic findings were seen in the sections of the deltoid muscle sample.

The prominent finding of our study was a perivascular infiltration of mononuclear cells with an increased number of CD8-positive T lymphocytes. The few previous reports on the pathology of the attack in ICLS are based on small amounts of tissues (muscle and/or skin) obtained by biopsy and do not describe perivascular infiltrates.^{8,9} Nevertheless, perivascular lymphoid infiltrates appear to be the main lesions in several organs of mice

treated with rIL-2 to induce a CLS.³ In these animals the endothelial cells surrounded by such an infiltrate present cytoplasmic vesicles or vacuoles and intact cell junctions like those detected in our patient. Although rIL-2-induced CLS presents a multiorgan involvement² and ICLS seems to be limited to the skin or skin and muscles, the pathology is similar in both situations- cytotoxic lymphocytes surrounding vesiculated endothelial cells. These data and our previous demonstration of an increase in IL-2 receptor-positive cells during attacks in ICLS⁴ suggest that in this situation, too, activated lymphocytes are probably the cause of the endothelial damage. The pathway leading to lymphocyte activation remains unclear. We detected the unusual presence of CD1a/S100-positive cells in the upper dermis and in the intravascular spaces. These cells are typical antigen-presenting elements, and their presence makes it possible to hypothesize that ICLS is triggered by an immune reaction that elicits a cytokine-dependent lymphocyte activation leading to fluid extravasation.

A final comment concerns the observation that in our patient, as in other patients with ICLS, mentation was preserved in this shock condition. We, like others,⁷ have been very impressed with this observation, which has never been explained. One might postulate that all the processes involved in the transfer of oxygen from red blood cells to the mitochondria could increase the effective tissue oxygen-diffusing capacity. Recently, Gonzales et al.,¹⁰ calculating this lumped parameter from the best fit on the average data observed during hypoxic and normoxic exercise in experimental animals,

were able to show its significant increase when the hematocrit was elevated to 60%.

REFERENCES

1. Clarkson B, Thompson D, Horwith M, Luckey H. Cyclical edema and shock due to increased capillary permeability. *Am J Med* 1960;29:193-216.
2. Rosestein M, Ettinghausen SE, Rosenberg SA. Extravasation of intravascular fluid mediated by the systemic administration of recombinant interleukin 2. *J Immunol* 1986;137:1735-42.
3. Fujita S, Puri RK, Yu ZX, Travis WD, Ferrans VJ. An ultrastructural study of in vivo interaction between lymphocytes and endothelial cells in the pathogenesis of the vascular leak syndrome induced by interleukin-2. *Cancer* 1991;68:2169-74.
4. Cicardi M, Gardinali M, Bisiani G, Rosti A, Allavena P, Agostoni A. The systemic capillary leak syndrome: appearance of interleukin-2-receptor-positive cells during attacks. *Ann Intern Med* 1990;113:475-7.
5. Mason DY, Cordell JL, Gaulard P, Tse AGD, Brown MH. Immunohistological detection of human cytotoxic/suppressor T cells using antibodies to a CD8 peptide sequence. *J Clin Pathol* 1992;45:1084-8.
6. Atkinson JP, Waldmann TA, Stem SF, Gelfand JA, MacDonald WJ, Meck LW, et al. Systemic capillary leak syndrome and monoclonal IgG gammopathy. *Medicine (Baltimore)*. 1977;56:225-39.
7. Teelucksingh S, Padfield PL, Edwards CRW. Systemic capillary leak syndrome. *Q J Med* 1990;75:515-24.
8. Cain JR, Bourgoignie JJ, Ahn YS, Schulz DR. Systemic capillary leak syndrome (SCLS): a rare cause of systemic edema [abstract]. *Kidney Int* 1986;29:181.
9. Johansson BR, Löfdhal CG. Ultrastructure of the microvessels in skeletal muscle in a case of systemic capillary leak syndrome. *Acta Med Scand* 1979;206:413-6.
10. Gonzales NC, Erwig LP, Painter CF III, Clancy RL, Wagner PD. Effect of hematocrit on systemic O₂ transport in hypoxic and normoxic exercise in rats. *J Appl Physiol* 1994;77:1341-8.

Gastroesophageal reflux and severe combined immunodeficiency

Andreas Boeck, MD, Rebecca H. Buckley, MD, and Richard I. Schiff, MD, PhD
Durham, N.C.

Background: Gastrointestinal and respiratory symptoms and failure to thrive not associated with infections or medications were noted in patients with severe combined immunodeficiency. **Objective:** The aim of our study was to determine the frequency of gastroesophageal reflux in patients with severe combined immunodeficiency.

Methods: We studied the case histories of 73 pediatric patients who had been treated at Duke University Medical Center for severe combined immunodeficiency between 1982 and 1995. Charts were reviewed for documentation of gastroesophageal reflux on the basis of clinical course and results of barium swallow, esophageal pH probe monitoring, or endoscopy. To compare the incidence of gastroesophageal reflux in patients with severe combined immunodeficiency to known high-risk populations, we additionally tabulated the underlying diagnoses in an age-matched group of patients who underwent Nissen fundoplication from 1990 to 1995.

Results: We found clinically significant gastroesophageal reflux in 15 of the 73 patients (20.5%), much higher than has been reported in the normal population (0.1% to 0.3%, $p < 0.001$). Of patients treated between 1990 and the present, 10 of 36 (27.7%) had significant gastroesophageal reflux compared with five of 37 patients (13.5%) in the previous years. Thus with greater recognition and improved methods for diagnosis, the observed incidence of gastroesophageal reflux has increased greatly. The clinical presentations were not different from those of patients with other well-documented underlying diagnoses. Seven of the 15 patients (46.6%) did not respond to medical treatment with antacids, H_2 -blockers, and prokinetic agents and underwent surgical treatment. Indications for surgery included persistent esophagitis, vomiting, pneumonia, and growth failure. **Conclusions:** The reason for the high incidence of gastroesophageal reflux in patients with severe T-cell disorders remains unclear. Considering the frequency of this association, early recognition and treatment is important to enable adequate nutrition and prevent damage to the esophagus and lungs. (*J Allergy Clin Immunol* 1997;99:420-4.)

Key words: Gastroesophageal reflux, severe combined immunodeficiency, Nissen fundoplication

Severe combined immunodeficiency (SCID) is an inherited disorder with impaired function of both the

Abbreviations used

GER: Gastroesophageal reflux

SCID: Severe combined immune deficiency

cellular and humoral immune systems.^{1,2} Patients with this disease have severe, recurrent, often life-threatening infections that become clinically manifest in early infancy, after they have lost protection by maternal antibodies.³ Although antimicrobial therapy is necessary to treat acute infections and may be life-saving, prolonged survival requires reconstitution of the immune system by bone marrow transplantation, sometimes preceded by myeloablation.⁴ Gastrointestinal symptoms such as vomiting, diarrhea, or malabsorption are usually attributed to either infections or medications, but in many children gastrointestinal symptoms could not be explained by these common causes. Additionally, some of the children had respiratory problems not caused by infectious pathogens.

During the evaluation of these patients for the cause of their symptoms, we noted that there appeared to be a much higher incidence of gastroesophageal reflux (GER) in patients with SCID compared with the normal population. GER is a common condition in infancy characterized by repetitive regurgitation of gastric content into the esophagus. Although many infants have minor degrees of reflux, about 1 in 300 to 1 in 1000 have significant reflux and associated complications.⁵ Decreased tone in the lower esophageal sphincter, anatomic changes in the esophagus, increased gastric acidity, and poor esophageal clearance are some of the pathophysiologic factors leading to esophageal damage.⁶ Relief of symptoms after fundoplication in refractory asthma points to a close correlation between GER and bronchoconstrictive diseases.^{7,8} Additionally, an increased incidence of GER has recently been reported in patients allergic to cow's milk.⁹ It is also evident that patients with acquired, secondary immunodeficiencies are another high-risk GER population because of opportunistic infections.^{10,11} To determine a possible characteristic pattern of GER-associated symptoms in our patients, which may help to differentiate patients with SCID from patients with other underlying major defects, three main features were compared: respiratory problems, gastrointestinal symptoms, and failure to thrive including poor feeding. Many of the patients improved with medical management of their GER, but many

From Duke University Medical Center, Department of Pediatrics, Division of Allergy and Immunology, Durham.

Supported in part by a grant of the MAX KADE Foundation and by grant 5R01AI128414 from the National Institute of Allergy and Infectious Diseases.

Received for publication June 25, 1996; revised Aug. 8, 1996; accepted for publication Sept. 10, 1996.

Reprint requests: Andreas Boeck, MD, Duke University Medical Center, Department of Pediatrics, Division of Allergy and Immunology, 365 Jones Building, Box 2898, Durham, NC 27710.

Copyright © 1997 by Mosby-Year Book, Inc.

0091-6749/97 \$5.00 + 0 1/1/77914

others required surgical correction to control their symptoms. Our data make clear that careful monitoring and management of symptoms not related to infections or medications is necessary to improve the clinical course of patients with SCID.

METHODS

We studied the case histories of all patients with SCID who were followed up by the Duke Division of Pediatric Immunology and were seen between 1982 and 1995 ($n = 73$). Charts were reviewed for documentation of vomiting, malabsorption, feeding problems, failure to thrive, and respiratory symptoms-in particular, aspiration pneumonia. GER was considered on the basis of the clinical course and results of barium swallow, if aspiration was observed, but otherwise confirmed by results of esophageal pH probe monitoring or endoscopy. Three patients underwent lung biopsy, and seven underwent endoscopy and biopsy. Histologic findings in the esophagus revealed nonspecific inflammation, similar to that seen in patients without immune deficiency. Because of their age, no patients had studies of esophageal motility. Nearly all patients were treated with antacids, prokinetic agents, and H_2 antihistamines in the hospital during their treatment for immune deficiency. Patients received supplemental parenteral nutrition if oral intake was determined to be inadequate.

Patients with evidence of severe *Candida* infection of the esophagus, as well as patients with infectious or medication-associated pneumonia, were excluded from analysis. However, patients with well-documented aspiration pneumonia were included. Because of a perceived increase in the incidence of GER in the past 5 years, the study period was also analyzed separately from 1982 to 1989 and from 1990 to 1995.

The overall incidence of GER in pediatric patients at Duke is not known. Therefore to compare the incidence of reflux in patients with SCID with that of known high-risk populations, we tabulated the underlying diagnoses in an age-matched group of patients who underwent Nissen fundoplication from 1990 to 1995. Data were obtained from the Pediatric Surgery database and included all patients who either did not respond to medical treatment or for whom a trial of medications was not considered appropriate ($n = 121$). These included patients with a variety of anatomic abnormalities (diaphragmatic and paraesophageal hernia, tracheoesophageal fistula, esophageal stricture and atresia, pyloric stenosis), functional abnormalities (esophageal dysmotility, dyskinesia, and myopathy), congenital disorders other than immune deficiency (congenital heart disease, inborn errors of metabolism, Down syndrome, and other chromosomal abnormalities, hydrocephalus, severe intracranial abnormalities), immaturity (preterm infants, intrauterine infections, parental drug abuse), infantile cerebral palsy class II to III, and cystic fibrosis. Children who were otherwise considered to be normal were also included.

RESULTS

As shown in Table I, we found a significantly increased incidence of GER in patients with SCID (20.5%), compared with that reported in the normal population (0.3% to 0.1%, $p < 0.001$).⁵ The mean age at diagnosis of GER was 16.6 months (range, 3 months to 5 years). In most patients the symptoms of respiratory distress and failure to thrive led to an initial diagnosis of SCID, and GER was suspected and diagnosed after referral. However, since these pa-

TABLE I. Incidence of GER in patients with SCID

	Observation period		
	Total period 1982-95	1982-89	1990-95
No. Patients with SCID	73	37	36
No. with GER	15 (21%)	5 (14%)	10 (28%)
No. undergoing surgery	7 (40%)	1 (20%)	6 (50%)

tients were referred because of their immune deficiency, this may not reflect the relative importance of reflux to their clinical course. The incidence of diagnosed GER was markedly higher in the years 1990 to 1995 compared with previous years, 1982 to 1989 (27.7% vs 13.5%). This finding may not be statistically significant because of the small number of patients. However, it is probable that recognition and diagnostic evaluation have improved in the past years, thus leading to a twofold higher incidence of confirmed GER compared with the earlier period; most likely, the true incidence from 1982 to 1989 was higher. On the basis of our findings, GER is to be expected in at least one of every five patients with SCID. Additionally, in the past 5 years markedly more children had a severe, complicated clinical course, did not respond to medical treatment, and thus underwent surgical intervention.

Table II shows the distribution of the three main symptoms of GER in patients with SCID. There was no consistent pattern of symptoms in our study population. The clinical presentation was heterogeneous with slight dominance of gastrointestinal problems: nine of 15 patients experienced frequent vomiting and esophagitis, of which three showed severe symptoms. Seven patients had recurrent respiratory problems; two of them had severe aspiration pneumonia. Though many of the patients had other respiratory symptoms, such as wheezing or cough, these symptoms were not used to support the diagnosis of GER because concurrent respiratory tract infections made it impossible to attribute them to reflux alone. Five children were suspected of having SCID because of poor feeding and failure to thrive. Of all children who underwent surgical correction at our institution, 6.6% were patients with severe immunodeficiency, despite the rarity of this diagnosis (Table III). GER causing symptoms and requiring surgical treatment was more common in children with SCID than in patients with functional abnormalities, cystic fibrosis, or asthma; and the number of children requiring surgery was equal to the number of premature infants. The majority of patients without SCID undergoing Nissen fundoplication had either anatomic abnormalities or congenital diseases, such as hydrocephalus and a variety of inborn errors of metabolism. Seven of 73 patients with SCID required surgery; it is not possible to determine the percentage of patients with any of the other diseases who underwent surgical correction because the preva-