

Insufficient numbers of biological replicates, and off-target effects, a well-known phenomenon in knockdown studies, may have played a role. Most of these studies obtained a significant reduction in FLG mRNA or protein expression (70% to 90%), but none of them reached complete absence of expression. We used genetically defined keratinocytes, completely deficient of FLG protein, in a 3D epidermal equivalent model. This provided a unique possibility to study the role of FLG in skin barrier defects, reported for both IV and AD. Thereby, knockdown-derived off-target effects were excluded, while potential compensatory mechanisms by naturally occurring FLG deficiency were taken into account.

In our study, barrier function was tested by the polar solutes LY and biotin as these are commonly used in comparable studies. Although alteration of the permeability for these low molecular weight tracers was not observed, this does not completely rule out alterations of FLG-deficient epidermis with respect to permeability for environmental molecules with other biophysical properties such as microbial or airborne antigens, or fragments thereof. As microbiome alterations (eg, *Staphylococcus aureus* colonization) may play a role in AD, the penetration of environmental stimuli may be the result of an interaction between microorganisms and genetic factors such as *FLG* mutations, as suggested in a recent study.⁸ Clearly, this is an interesting area for future research.

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Heterotaxy syndrome with and without spleen: Different infection risk and management



To the Editor:

Heterotaxy syndrome (HS) is a rare multiorgan disorder characterized by congenital heart defects (CHDs) and abnormal arrangement of the internal organs of chest and abdomen. Asplenia, polysplenia, hyposplenia, or ectopic spleens are frequently observed.¹ HS is divided into 2 forms: right atrial isomerism, which is associated with worse prognosis compared with the second form: left atrial isomerism. HS with right atrial isomerism is associated in more than 50% of the cases with primary asplenia and is considered the most frequent form, occurring approximately in 1 in 10,000 to 40,000 births. Left atrial isomerism is more frequently associated with polysplenia. Early surgical management of CHD has improved the life expectancy of children with HS, but lifelong care by a cardiologist is essential. Patients with HS are at higher risk for severe community-acquired bacterial infections than are patients with similar complex CHD. Sepsis, mostly due to pneumococcus, is lethal in 31% of the cases.² A recent study has demonstrated that low frequency of IgM memory B cells correlates with the risk of severe bacterial infection in HS.³ Because IgM memory B cells need the spleen for their generation, survival,⁴ and function,⁵ we asked the question of whether the risk of infections depends on the presence of the spleen in HS.

Our institute is a referral center for HS. Over the last 30 years, 123 patients underwent specific conservative and surgical management early after birth. During our follow-up period, 10 patients died (8%), 4 of which were asplenic. Two of the asplenic patients died because of sepsis, one at age 1 month and half and the other at age 6 months. In our institute, children born with HS undergo specific abdominal ultrasound scan to assess the presence, morphology, dimension, and location of the spleen. Patients are considered asplenic when this procedure fails to find splenic tissue. In the first year of life, all patients with HS receive the vaccines recommended by the Italian Ministry of Health for infants (see Table E1 in this article's Online Repository at www.jacionline).

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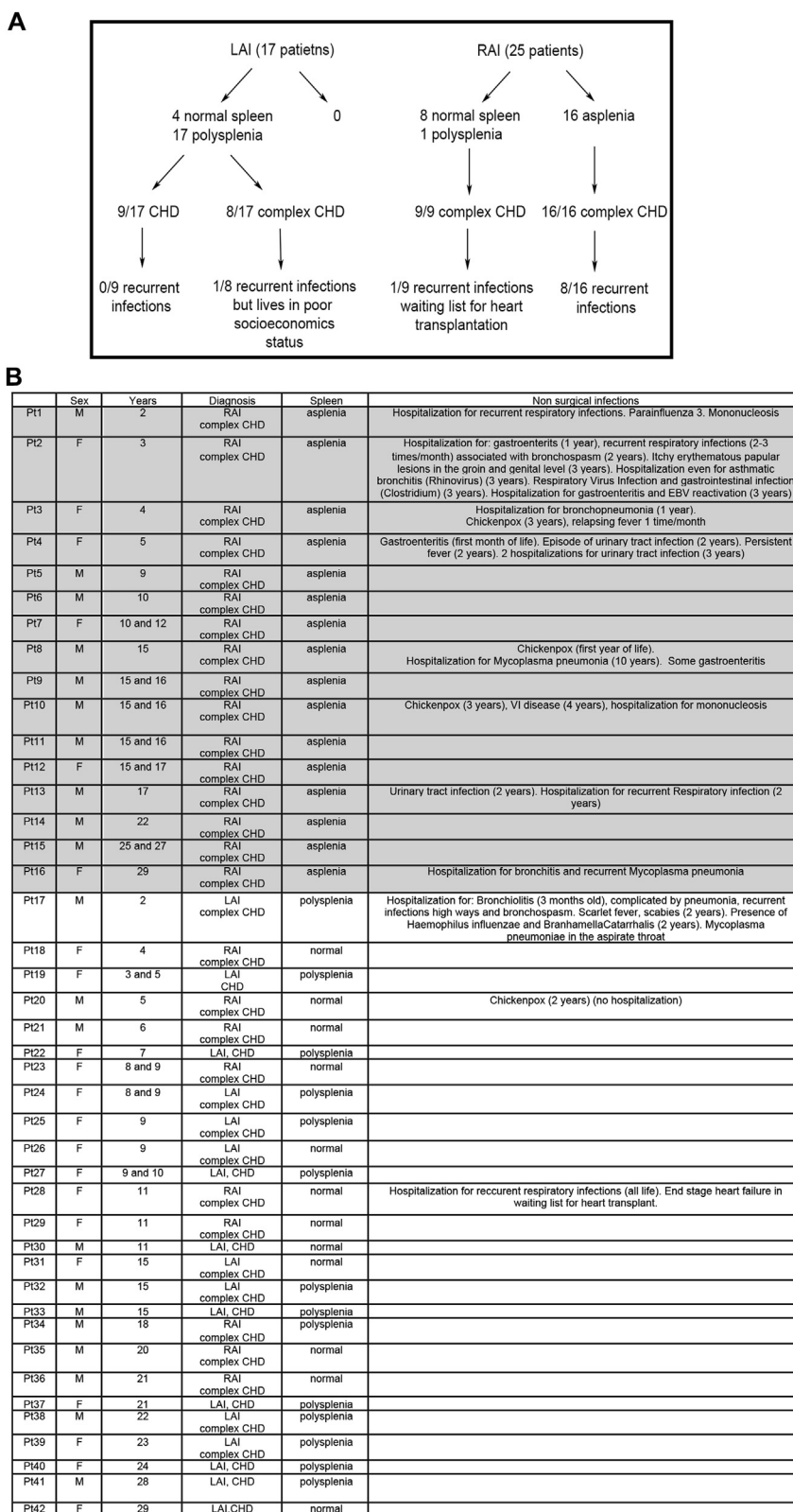


FIG 1. A and B, Detailed clinical information on each patient. *LAI*, Left atrial isomerism; *RAI*, right atrial isomerism.

org), including the glycoconjugated vaccines against *Haemophilus influenzae*, pneumococcus and meningococcus C. Asplenic children with HS also receive continuous antibiotic prophylaxis according to the current guidelines for patients without spleen.⁶

To demonstrate whether the absence of the spleen determines increased susceptibility to infections in HS, we reviewed the frequency of infection and absolute numbers of memory B cells in 42 children with HS, 16 of whom were asplenic. Patients'

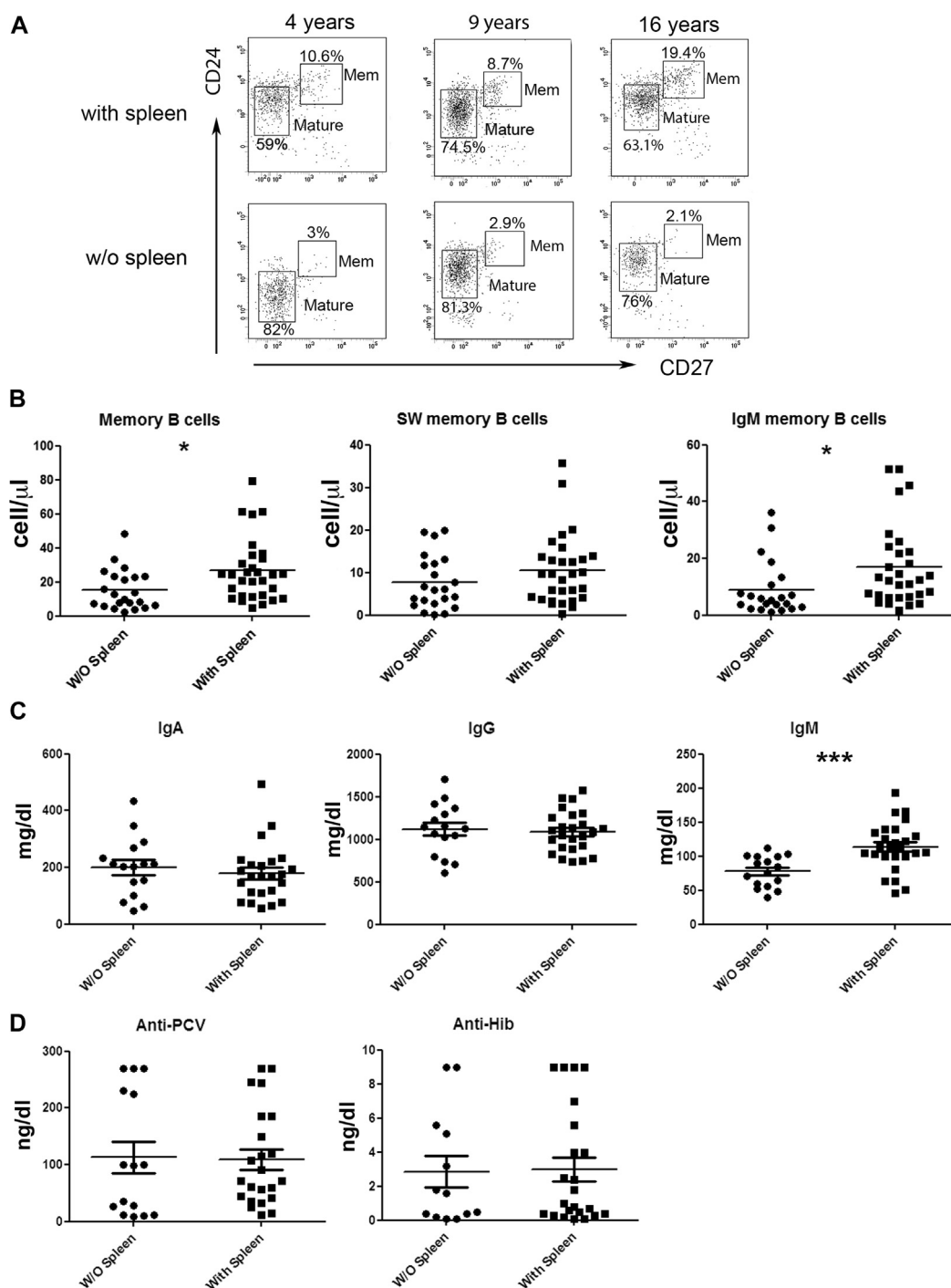


FIG 2. **A**, Dot plots show the percentage of mature and memory B cells gated on the CD19⁺ population. **B**, Absolute numbers of total memory, IgM, and switched memory B cells in the PB of patients without (n = 26) and with spleen (n = 16). **C**, Immunoglobulin level in the PB of patients without and with spleen. **D**, Anti-PCV and Anti-Hib antibody levels after immunization. *Hib*, *Haemophilus influenzae* type B vaccine; *PCV*, pneumococcal conjugate vaccine. *t* test, **P* < .05; ****P* < .001.

characteristics and clinical history are reported in Fig 1, A and B. Asplenic children with HS had a significantly higher incidence of infections that required hospitalization (50%, 8 of 16) compared with patients with HS with a normal spleen or polysplenia (8%, 2 of 26 patients; *P* < .05, Fisher exact test). Thanks to regular antibiotic prophylaxis, invasive bacterial diseases were rare, but

infections of the respiratory, gastrointestinal, and urinary tract were reported, mostly occurring during the first 5 years of life. EBV infections led to hospitalization in 3 cases. Only 2 of the HS patients with spleen, who have no indication for antibiotic prophylaxis, had recurrent infections of bacterial origin that needed hospitalization. One of them lives in poor socioeconomic

conditions and is highly exposed to community-acquired infections; the second child has a very severe hemodynamic instability and is in the waiting list for heart transplantation.

To determine the frequency and absolute numbers of total, IgM, and switched memory B cells, peripheral blood was collected during a follow-up visit and cells were stained for expression of CD19, CD24, CD27, CD38, and IgM (BD Biosciences, Milan, Italy).

The absence of the spleen was associated with the reduction of memory B cells. Dot plots show the percentage of mature ($CD24^+CD27^-$) and memory ($CD24^+CD27^+$) B cells in patients with or without spleen at 3 different ages (4, 9, and 16 years) (Fig 2, A). The frequency (not shown) and absolute numbers of total memory B cells ($P = .01$) and of IgM memory B cells ($P = .03$) were significantly lower in asplenic HS children than in those with a normal spleen or polysplenia. The number of switched memory B cells did not differ between the 2 groups (Fig 2, B). We also measured immunoglobulin levels (IgA/IgG/IgM) in both groups. No difference was found in IgA and IgG levels, whereas IgM levels were significantly reduced in asplenic patients with HS ($P = .0009$) (Fig 2, C). Specific IgG antibodies against vaccine antigens (PCV, pneumococcal polysaccharides, and Hib, *Haemophilus influenza*) were not different in patients with or without spleen (Fig 2, D). In the asplenic group, there was no difference in the severity of the cardiac disease between patients with or without infections. All immunological parameters were comparable with the exception of total IgG concentrations that, although always in the normal range, were significantly higher in patients without infections (data not shown).

The absence of the spleen is a well-known risk factor for severe bacterial infections especially with encapsulated microorganisms.⁷

Accordingly, a recent review of the literature showed that among patients with HS not receiving antibiotic prophylaxis the cases of bacteremia occurred mostly in asplenic patients (78% vs 21%).

Mortality rates were similar for HS patients with or without spleen.⁸ In our cohort because of antibiotics prophylaxis no systemic infection by encapsulated bacteria occurred and none of the patients died of sepsis. The spleen is a blood filter and lymphatic organ, necessary for the response to pneumococcal polysaccharides. Because of its filtering function in combination with a highly organized and large (about one-fourth of the body's lymphocytes are in the spleen) lymphoid compartment, the spleen plays a fundamental role in immune responses to blood-borne antigens.

The spleen, together with the thymus, is the phylogenetically oldest lymphoid organ. In evolution, the bone marrow is a late acquisition, first described in amphibians, and lymph nodes are even more recent lymphoid structures, first found in birds. It has been hypothesized that IgM memory B cells may be remnants of a primitive immune system that has been conserved in mammals to guarantee the first line of defense, whereas switched memory B cells are produced by the adaptive immune response.⁹ It has been shown that although both IgM and switched memory B cells are located in the spleen, only IgM memory B cells are significantly reduced in both primary and secondary asplenic patients.⁴

Our study on patients with HS shows that the lack of spleen and significant reduction in IgM memory B cells are associated with recurrent infections mostly of the respiratory and gastrointestinal

tracts. The observation that asplenic patients with HS, who are protected from encapsulated bacterial infection by antibiotic prophylaxis, still suffer from recurrent infections caused by other bacteria, fungi, and viruses suggest that the spleen and IgM memory B cells play an important role as first-line defense against all types of infection.

In our cohort, infections were more frequent in the first 4 years of life as observed before in splenectomized patients.¹⁰ Our observation confirms that the spleen and IgM memory B cells are particularly important in the first years of life, before the complete maturation of the adaptive immune response.

Our study demonstrates that at the time of diagnosis of HS, it is important to investigate the splenic condition of these neonates. Early treatment with prophylactic antibiotics⁶ and specific immunization programs effectively prevent severe bacterial infections and significantly reduce mortality in asplenic patients with HS. This group of patients remains, however, at a higher risk for other types of infections than do patients with spleen.

This study is part of our institute's health directory: good clinical practice program titled "Cardiogenetic pathway for children with heart diseases."

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TABLE E1. Immunization schedule according to the Italian Ministry of Health (http://www.salute.gov.it/portale/temi/p2_6.jsp?lingua=italiano&id=648&area=Malattie%20infettive&menu=vaccinazioni)

Vaccine	Birth	Age									Every 10 y
		3 mo	5 mo	6 mo	11 mo	13 mo	15 mo	5-6 y	11-18 y	>65 y	
DTP		DTPa	DTPa	DTPa	DTPa	DTPa	DTPa	DTPa	dTpa		dT
IPV		IPV	IPV	IPV	IPV	IPV	IPV	IPV			
Hepatitis B	HBV*	HBV	HBV	HBV	HBV	HBV	HBV	HBV			
Hib		Hib	Hib	Hib	Hib	Hib	Hib	Hib			
PCV		PCV-13	PCV-13	PCV-13	PCV-13	PCV-13	PCV-13	PCV-13			
MMR							MMR	MMR	MMR		
Men C							Men C		Men C		
HPV									HPV (3 doses)		
CP									CP (2 doses)		
Influenza										Influenza	

The mandatory vaccines are in boldface.

CP, Chickenpox; dT, diphtheria tetanus vaccine; DTPa, diphtheria-pertussis (whooping cough)-tetanus vaccine; HBV, hepatitis B virus vaccine; Hib, *Haemophilus influenzae* type B vaccine; HPV, human papilloma virus; IPV, inactivated polio vaccine; MenC, Meningococcal C Conjugate vaccine; MMR, mumps-measles-rubella vaccine; PCV, pneumococcal conjugate vaccine.

*In newborns of affected mothers.