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Chronic Aichi virus infection in a patient with X-linked agammaglobulinemia.

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To the Editor,

X-linked agammaglobulinemia (XLA) is caused by mutations in *BTK*, the gene encoding Bruton's tyrosine kinase. BTK is critical for human B cell development and maturation, and hemizygous loss-of-function (LOF) mutations result in peripheral B cell lymphopenia, rudimentary tonsils and lymph nodes, and severely reduced to absent levels of serum immunoglobulins (Ig). Affected boys present in infancy with recurrent and often life-threatening respiratory tract and skin infections with encapsulated bacteria. Gastrointestinal infections with pathogens such as *Giardia lamblia* are also common, and they can manifest autoimmunity and autoinflammation (1,2). XLA patients also have extreme susceptibility to viral infections, especially with Enteroviruses, which can cause severe central nervous system (CNS) disease (3). The risk of Enteroviral infection is particularly high in patients who experience a diagnostic delay. Chronic Norovirus infection of the gut is another problematic viral infection (4–6), while Astrovirus and Cache Valley virus have been described to cause progressive encephalitis in XLA patients (7,8).

Here we describe a boy with XLA due to a deleterious mutation in *BTK* (c82C>T, p. R28C), diagnosed at the age of three years. At diagnosis, he had near absent B cells (7 cells/µL, <1% of total lymphocytes) and markedly reduced immunoglobulin levels (IgG 0.77 g/L, IgM 0.09 g/L and IgA 0.03 g/L). Between the age of six and thirteen years, following a holiday in northern Italy, he developed a fever of unknown origin, intermittent bloody diarrhea, generalized lymphadenopathy, progressive hepatosplenomegaly with abdominal distension, progressive nephromegaly and refractory temporal lobe epilepsy (detailed clinical history can be found in this article's supplemental data). Blood analysis showed progressive pancytopenia, progressive elevation of liver enzymes, and chronic kidney failure with a glomerular filtration rate as low as 50 ml/min/1.73m^2, as measured by ^{51}Cr-EDTA clearance. On ultrasound multiple hypodense focal lesions were evident in the liver, spleen and kidneys, which were enlarged. Histological examination of a liver biopsy specimen demonstrated severe chronic hepatitis with initial fibrosis. Kidney histology showed pronounced diffuse interstitial oligoclonal cytotoxic T cell infiltrates with changing TCR

Vβ repertoires on sequential biopsies (Fig. S1 in this article's supplemental data). Colon biopsy showed chronic colitis. The patient developed severe growth delay. At the age of thirteen years he underwent splenectomy, which resulted in resolution of his abdominal discomfort and pancytopenia. This was suggestive of hypersplenism as the underlying cause of cytopenia.

No microbial agent could be grown from or detected by routine diagnostics including PCR in blood, urine, cerebrospinal fluid (CSF) or biopsied tissues, and multiple treatments, such as antimicrobial therapies *ex juvantibus*, elevation of the Ig supplementation dose, and corticosteroids, were ineffective in controlling fever, diarrhea, vomiting, progressive kidney and liver anomalies and convulsions. Only during empirical treatment with liposomal amphotericin B (on the suspicion of *Leishmania* infection) did the patient become afebrile, while antibiotics and nitazoxanide gave no benefit. Immunophenotyping of his peripheral blood showed an increase in ~~proportions~~ absolute number and percentage of $CD8^+$ T cells, mostly displaying a memory phenotype (i.e. high numbers of $CD45RA^-CCR7^+$ central and $CD45RA^-CCR7^-$ effector memory cells and reduced numbers of $CD45RA^+CCR7^+$ naïve and terminally differentiated $CD45RA^+CCR7^-$ effector memory cells), compared to healthy donors and a control XLA patient. Furthermore, his memory $CD8^+$ T cells displayed increased expression of markers of activation (HLA-DR, CD38 and HLA-ABC) and exhaustion/senescence (CX3CR1, CD95, CD57; Fig. 1). In contrast, the control XLA patient had decreased $CD8^+$ and increased $CD4^+$ T cells, with an increased proportion of naïve and terminally differentiated effector memory cells and corresponding decreased proportions of central and effector memory cells compared to healthy controls. Moreover, expression of activation and exhaustion/senescence markers on $CD8^+$ T cells from the control XLA patient was comparable to or lower than that of healthy controls (Fig. 1, D-I). This is in agreement with the subtle defects previously described in T cell maturation in XLA patients (9,10).

RNA-sequencing is a powerful tool in the field of infectious diseases, as it allows for the detection of viral RNA in pathological tissue samples (11,12), and we therefore applied it in a snap-frozen sample from a

kidney biopsy performed at twelve years of age in our patient. The whole-tissue RNA-seq was found to be positive for Aichi virus (AiV1), and subsequently samples of liver, spleen, urine , CSF and sputum were also found positive for AiV1 on PCR. AiV1 is a Kobuvirus of the Picornaviridae family and causes self-limiting gastroenteritis in immunocompetent humans. It was first isolated in 1989 during a gastroenteritis outbreak associated with the consumption of raw oysters in Aichi prefecture, Japan, and was later genetically characterized (13). AiV1 is a common contaminant of water ponds, sewages and shellfish all around the world and its seroprevalence reaches almost 100% by the age of 30 years in all published studies (14–19). Despite the high prevalence of anti-AiV1 antibodies in children and adults, AiV1 is found rarely in stool samples from patients with acute sporadic or epidemic gastroenteritis and, on average, is isolated in fewer than 1% of cases (14,20). However, the prevalence of AiV1 in stool specimens of HIV-positive patients is higher, probably indicating an opportunistic behavior in patients with underlying T cell defects (20,21).

Enteroviruses, like AiV1, belong to the Picornavirus family, and are a cause of chronic infection in XLA patients. Chronically infected patients commonly develop meningoencephalitis, sometimes showing the acute flaccid paralysis that is characteristic of poliovirus infections, but arthritis, hepatitis, dermatomyositis, polyradiculitis and myocarditis have also been described (3). Of note, CSF is often normal, and both culture and PCR for Enteroviruses yield a high false negative rate. Consequently, the gold standard for diagnosis is to perform PCR or NGS techniques on a brain biopsy (3). Our patient presented with severe multi-organ involvement. A classical microbe-isolation approach, including the use of pan-bacterial PCR, was insufficient to identify the etiologic agent, while RNA-seq obviated the limitations of standard methods in identifying AiV1. Chronic Norovirus gastrointestinal infection is another difficult-to-treat infectious complication of XLA, sometimes severe enough to prompt hematopoietic stem cell transplantation (HSCT) (4,6).

Although well described, the susceptibility of XLA patients to chronic viral infections is unexpected and ill-explained. A defect in $CD4^+$ T cell maturation has been described in XLA, with reduced $CD4^+CD45RO^+$ and $CD4^+CD45RO^+CXCR5^+$ memory T cells and impaired delayed cutaneous hypersensitivity reaction (9,10,22). Together, these subtle defects could contribute to susceptibility to viral and opportunistic infections in these patients. Various therapeutic strategies have attempted to control and/or eradicate Enterovirus and Norovirus in XLA patients, mostly with little success. High dose Ig substitution is recommended, and local administration (intrathecal or enteral) has been described to yield better outcomes than parenteral infusion alone (3,6). Antiviral drugs such as cidofovir, ribavirin and interferon (IFN)-α or -β have mostly been ineffective (3,6). Nitazoxanide, a broad-spectrum antimicrobial agent with antiviral and Norovirus replication-inhibiting properties in vitro, has been used in immunodeficient patients with chronic Norovirus infection with mixed results (5,6). Pleconaril (an anti-Picornavirus medication) was the most frequently used antiviral in chronic enteroviral meningitis, but is no longer available, while other direct-acting antiviral molecules are currently under development (Vapendavir, Pocapavir and ViroD7000) (3). Also, itraconazole and fluoxetine showed anti-Enterovirus properties in vitro, and successful treatment of chronic enteroviral meningitis with fluoxetine has been reported in a XLA patient (3,23). Antiviral effects of immunosuppressive regimens in transplanted and other immunodeficient patients have been reported, specifically the anti-Cytomegalovirus and anti-Norovirus efficacy of the mTOR (mechanistic target of rapamycin) inhibitors sirolimus and everolimus (6,24–26). Recently, amphotericin B was found to be a potent inhibitor of two different Enteroviruses, in addition to its known anti-fungal indication and the described anti-parasitic and anti-viral effect on *Leishmania*, vescicular stomatitis virus, herpes simplex virus, Sindbi virus, human immunodeficiency virus and others (27). However, no specific data is available for AiV1.

In the present patient, various courses of treatment were attempted after Aichivirus detection and confirmation. High doses of Ig were poorly tolerated from a neurological point of view; nitazoxanide

treatment gave no results, and itraconazole therapy was discontinued after a few days due to hepatic toxicity. Only liposomal amphotericin B resulted in a temporary afebrile status, and a new trial with Ambisome is envisaged. We tried to introduce fluoxetine in increasing doses with some difficulty due to adverse effects. Immunosuppression with low dose tacrolimus was also attempted to reduce the hyperactivation of his $CD8^+$ T cells (observed both in blood and as infiltrates in the kidney biopsies). Many studies of patients suffering chronic viral infections showed that CD8+ T cells prematurely undergo exhaustion and senescence, thereby becoming dysfunctional due to constant activation by persistent viral stimuli (28–31). This was also observed in our patient. Given the severity of his presentation and progressive kidney failure due to diffuse T cell infiltrate in the renal interstitium, HSCT using the patient's HLA-matched sister as a donor is being considered. In conclusion, we describe AiV1 as the cause of severe chronic multi-organ disease and chronic kidney failure in a patient with XLA, and we invite to consider HSCT as a treatment option.

Compliance with Ethical Standards

Author IM has received a CSL Behring grant paid to Institution. The authors have no further conflicts of interest to disclose.

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent was obtained from all individual participants included in the study.

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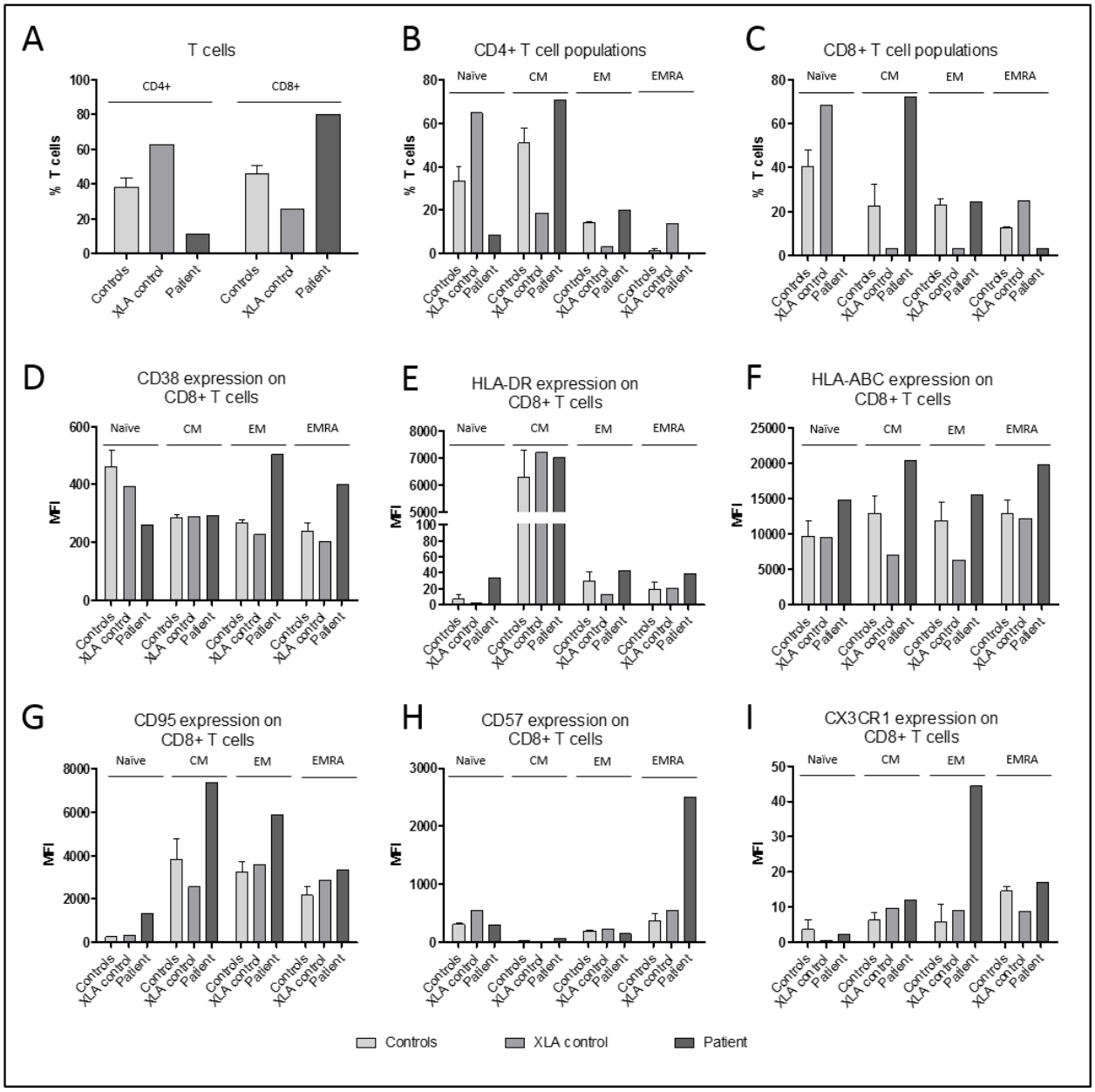

Fig. 1. CD8+ T cells in the proband XLA patient are skewed towards a memory phenotype with evidence of exhaustion and/or senescence. PBMCs from healthy controls, an XLA control and our XLA patient were labeled with mAbs against CD4, CD8, CD45RA, CCR7, CD38, HLA-DR, HLA-ABC, CD95, CD57, and CX3CR1. Proportions of (A) $CD4^+$ and $CD8^+$ T cell populations, as well as subsets of naïve ($CD45RA^+CCR7^+$), central memory (CM, $CD45RA^-CCR7^+$), effector memory (EM, $CD45RA^-CCR7^-$), and terminally differentiated effector memory cells expressing CD45RA (EMRA, $CD45RA^+CCR7^-$), (B) $CD4^+$, and (C) $CD8^+$ T cells were delineated. Differential expression of the activation markers CD38 (D), HLA-DR (E), and HLA-ABC (F), as well as the exhaustion/senescence markers CD95 (G), CD57 (H) and CX3CR1 (I) on naïve, T_{CM}, T_{EM} and T_{EMRA} $CD8^+$ T cells were determined. Values represent the geometric MFI.

Figure 1

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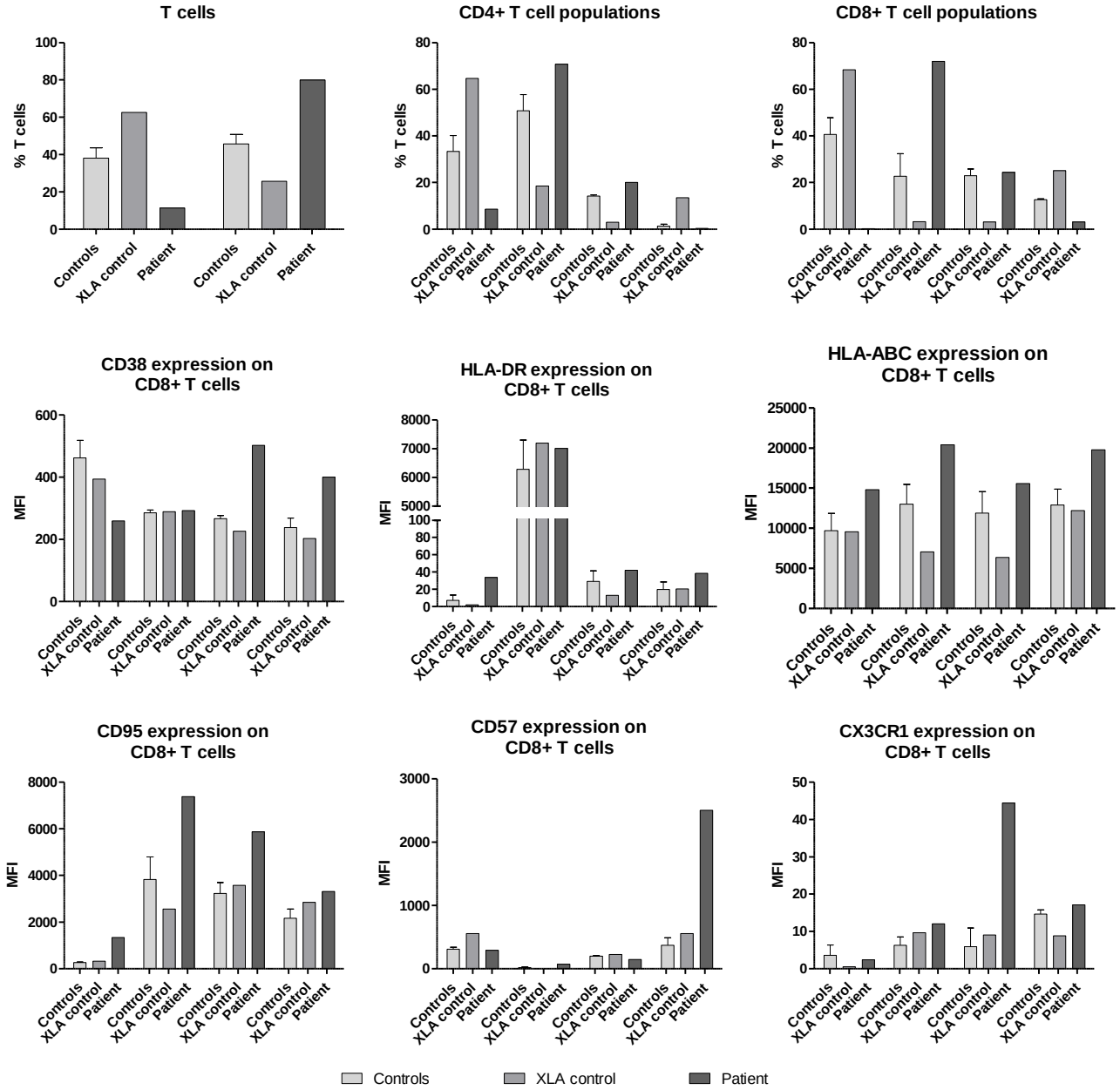

Figure S1

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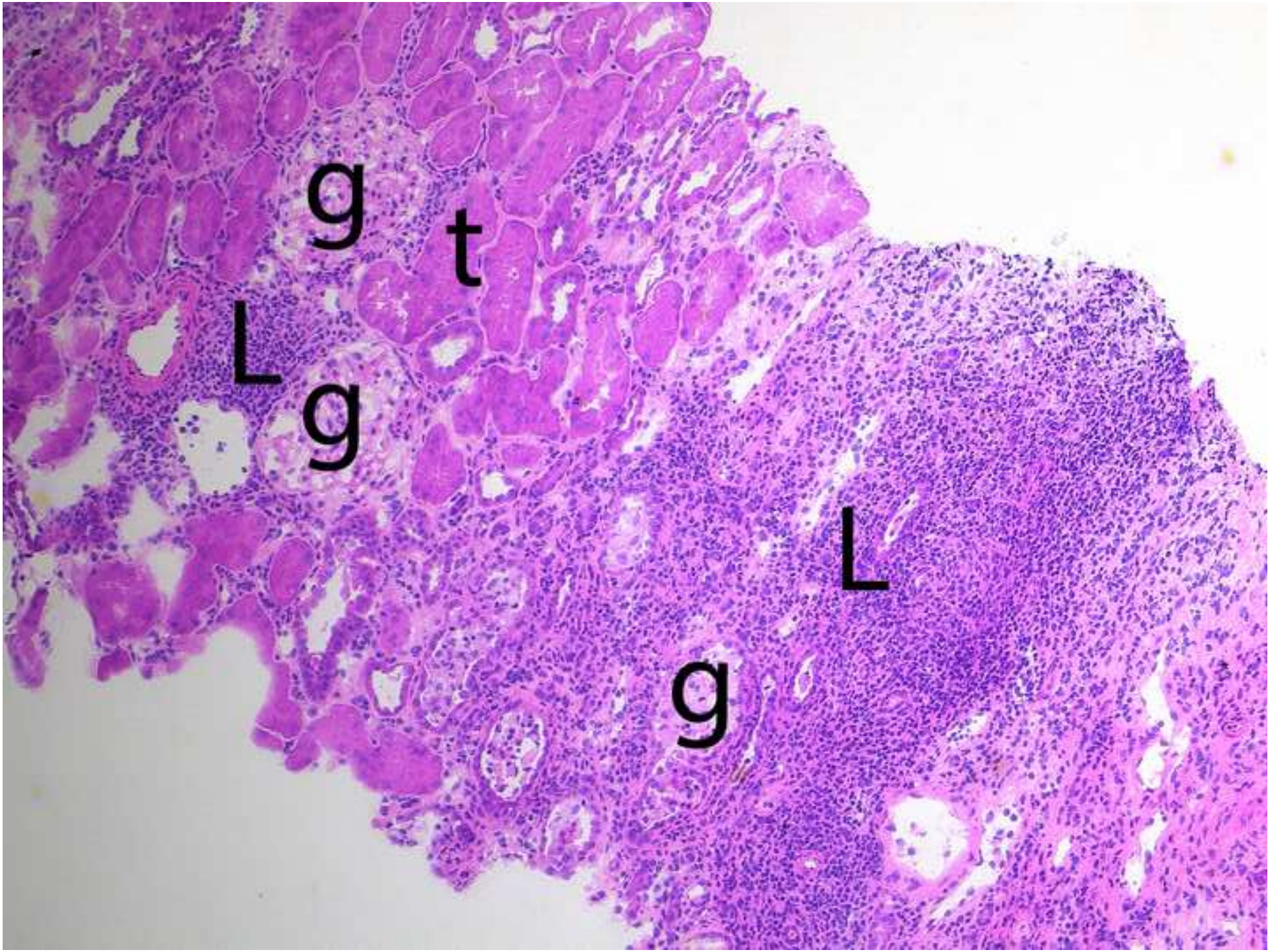

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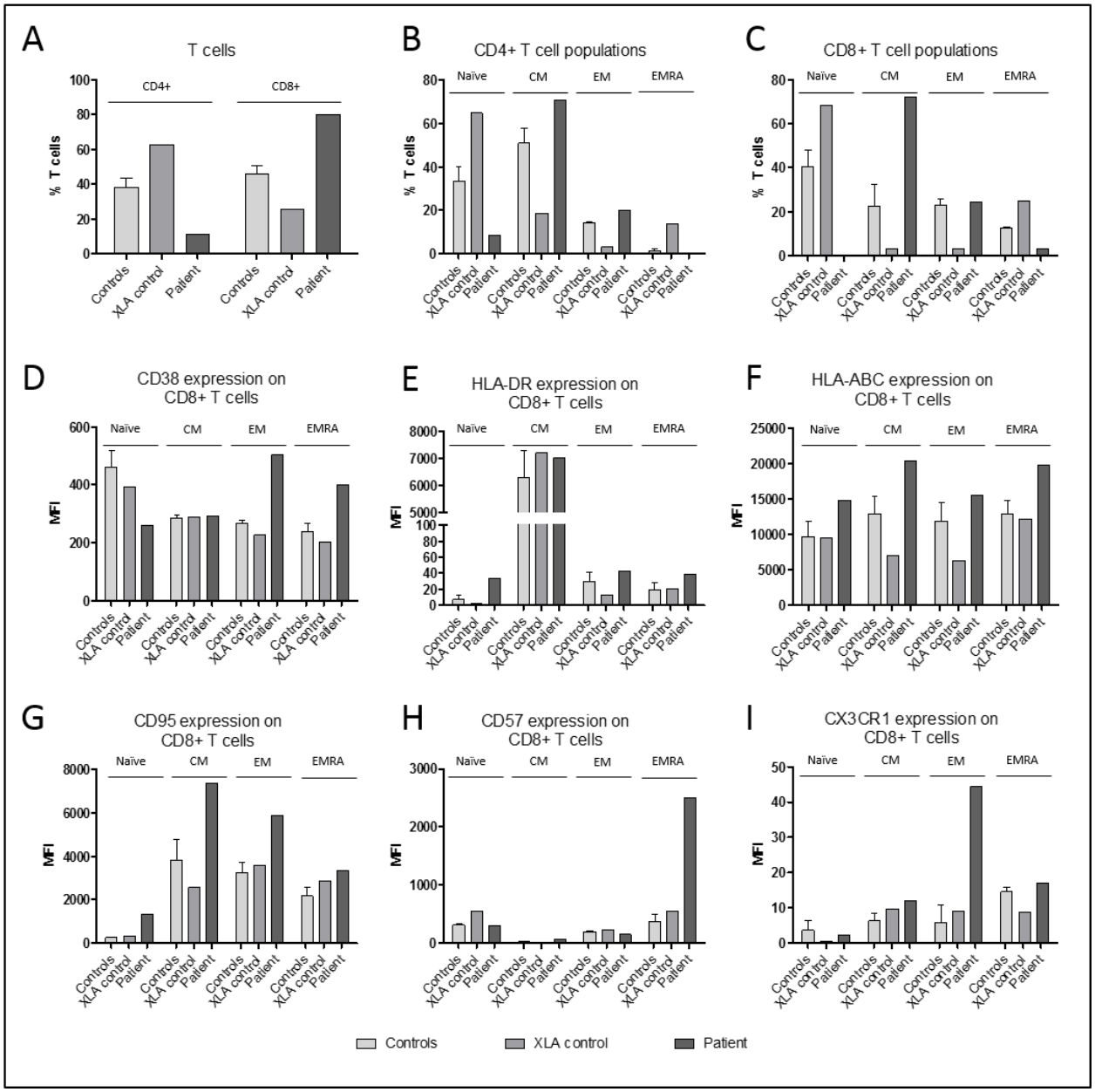

Fig. 1. CD8+ T cells in the proband XLA patient are skewed towards a memory phenotype with evidence of exhaustion and/or senescence. PBMCs from healthy controls, an XLA control and our XLA patient were labeled with mAbs against CD4, CD8, CD45RA, CCR7, CD38, HLA-DR, HLA-ABC, CD95, CD57, and CX3CR1. Proportions of (A) $CD4^+$ and $CD8^+$ T cell populations, as well as subsets of naïve ($CD45RA^+CCR7^+$), central memory (CM, $CD45RA^-CCR7^+$), effector memory (EM, $CD45RA^-CCR7^-$), and terminally differentiated effector memory cells expressing CD45RA (EMRA, $CD45RA^+CCR7^-$), (B) $CD4^+$, and (C) $CD8^+$ T cells were delineated. Differential expression of the activation markers CD38 (D), HLA-DR (E), and HLA-ABC (F), as well as the exhaustion/senescence markers CD95 (G), CD57 (H) and CX3CR1 (I) on naïve, T_{CM}, T_{EM} and T_{EMRA} $CD8^+$ T cells were determined. Values represent the geometric MFI.