

High-throughput analysis revealed the unique immunoglobulin gene rearrangements in plasmacytoma-like post-transplant lymphoproliferative disorder

Post-transplant lymphoproliferative disorder (PTLD) is a severe complication of solid organ and haematopoietic stem cell transplantation (SOT, HSCT), which occurs in 1–20% of transplant recipients.¹ The majority of PTLD cells are of the B-cell type, but <5% of PTLD cells are plasma-cell type, including plasmacytoma-like lesion and plasma cell myeloma.¹ Although their worse prognosis than non-PTLD myeloma is clinically significant, only case reports and registry studies have been available.² In the present study, we describe the first detailed analysis of plasmacytoma-like PTLD.

A 2-year-old boy, who had X-linked inhibitor of apoptosis protein (XIAP) deficiency (Fig S1), developed Epstein–Barr virus-negative plasmacytoma-like PTLD 7 months after HSCT.³ His clinical presentation is described in the supplemental data. The histopathological analysis of lymph nodes showed that the lymph node architecture was disrupted by diffusely proliferating dysplastic plasma cells, which were positive for cluster of differentiation (CD)138 and negative for CD20 (Fig 1A–C). The cytoplasm was positive for immunoglobulin (Ig)G and weakly positive for IgA with lambda light-chain restriction (Fig 1D–G). Short tandem repeat analysis showed recipient chimerism in the lymph nodes, whereas the bone marrow showed donor chimerism, except for 10% of the PTLD cells (Fig S2). The karyotype was normal. To identify secondary genetic events, whole exome sequencing was performed using paired lymph node cell DNA and control DNA from the recipient. After filtering with donor-specific variants due to few contaminations of donor cells in the lymph node, nine tumour-specific variants were identified (Table SI). Somatic mutations of rho-associated coiled-coil-containing protein kinase 1 (*ROCK1*) and cell division control protein 42 homologue effector protein 1 (*CDC42EP1*) involved in the Rho signalling pathway were identified. In addition, a somatic mutation of lysine methyltransferase 2C (*KMT2C*), which regulates lysine methylation, was identified. Interestingly, activation of the Rho signalling pathway and mutation of *KMT2C* are known to be associated with non-PTLD myeloma development.^{4,5} We also evaluated germline variants, and identified a variant of breast cancer type 2 susceptibility protein (*BRCA2*) (homozygous p.K322Q), described as a variant of uncertain significance.⁶ However, we could not identify other possibly pathogenic variants. Malignancy has not been described in XIAP deficiency.

Next, to evaluate the cells of origin and clonal variation, we performed high-throughput sequencing of Ig heavy- and light-chain genes using total RNA from lymph node cells and peripheral blood mononuclear cells (ImMunoGeneTics; iRepertoire Inc., Huntsville, AL, USA). Repertoire analysis revealed a clonal expansion of Ig heavy variable 3-33 (*IGHV3-33*)/Ig heavy diversity 7-27 (*IGHD7-27*)/Ig heavy joining 5 (*IGHJ5*) in Ig heavy locus (*IGH*) and Ig lambda variable 1-47 (*IGLV1-47*)/Ig lambda joining 3 (*IGLJ3*) in Ig lambda locus (*IGL*) (Fig 2A, Fig S3). This clone was also detected in peripheral blood, suggesting haematogenous metastasis. Most constant regions consisted of Ig heavy constant gamma 4 (*IGHG4*), but a small fraction consisted of *IGHG1*, *IGHG3*, Ig heavy constant alpha 2 (*IGHA2*), Ig heavy constant epsilon (*IGH E*) and Ig heavy constant mu (*IGHM*), indicating clonal heterogeneity due to class switch recombination (CSR) (Fig 2B). In this clone, multiple intraclonal variants were identified, most likely due to somatic hypermutation (SHM) (Fig 2C,D). On the other hand, the frequencies of SHM of PTLD cells were significantly reduced with 1.3 ± 0.9 and 1.7 ± 1.1 mutations on average in *IGHV* and *IGLV*, respectively, compared with that of peripheral B cells from the patient or a normal control (Fig 2E). Non-PTLD myeloma cells, by contrast, have higher frequencies of SHMs than those of other B-cell malignancies, with 8–9% on average.^{7,8} These findings indicate that the PTLD cells were found to originate from germinal centre B cells exposed to on-going CSR but few SHMs, and committed to be plasma cells (Fig S4). Primary lymph node PTLD fits this model.

The limited SHMs can cause a defective peripheral B cell tolerance checkpoint, and the production of autoantibodies.⁹ Indeed, at the same time as the onset of PTLD, our patient developed autoimmune diseases including autoimmune bullous disease, autoimmune haemolytic anaemia (AIHA), immune thrombocytopenia (ITP) and autoimmune neutropenia (Fig 1H,I). Serological tests showed markedly elevated levels of autoantibodies, which were restricted in the lambda light-chain (Table SII, Fig S5). These findings were consistent with the lambda light-chain restriction of PTLD cells, suggesting that multiple autoantibodies produced by PTLD cells led to multiple autoimmune diseases. The expanded clone did not show an autoimmune-associated repertoire, such as *IGHV4-34*.¹⁰ The increased length of complementarity-determining region 3 was not found, which is

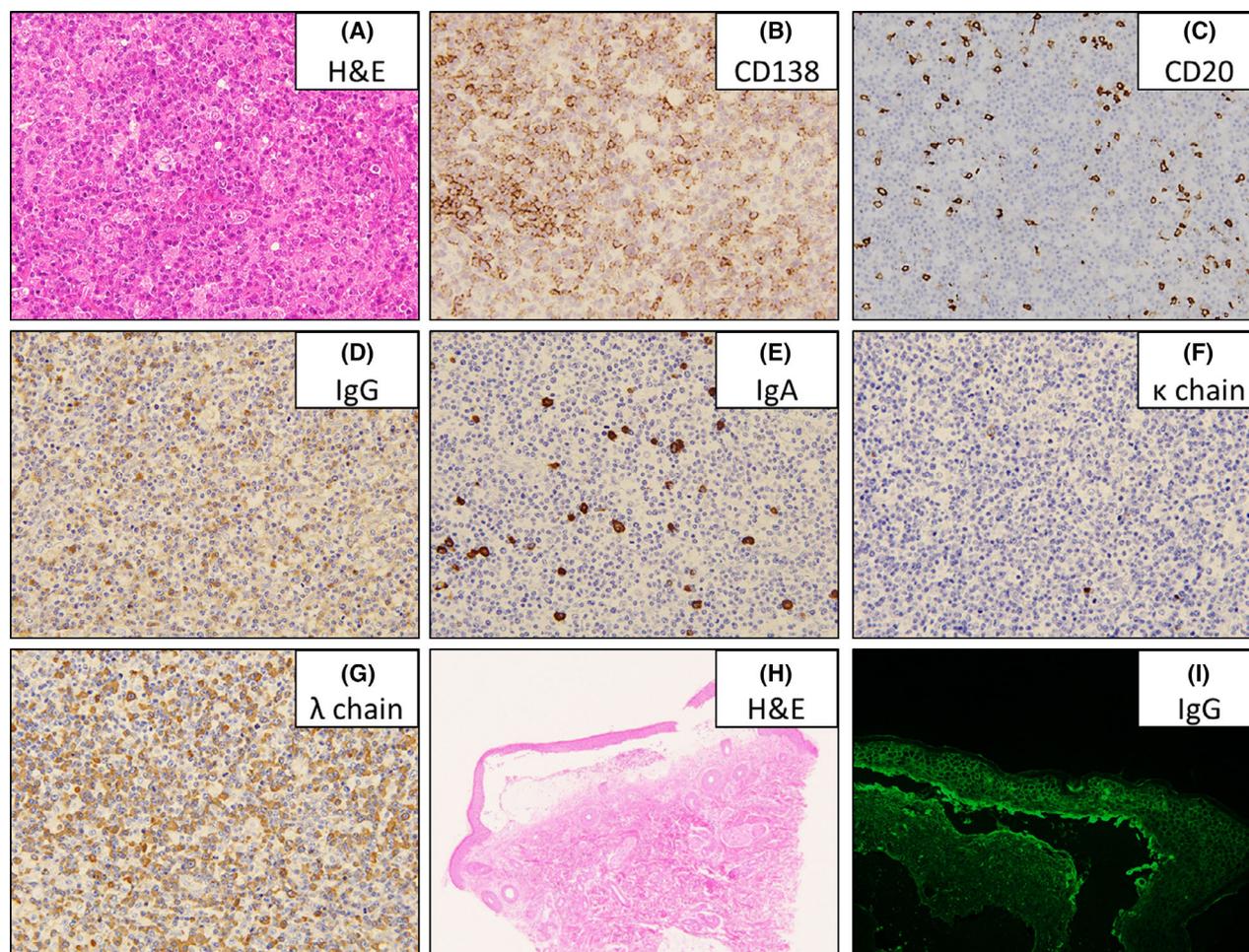


Fig 1. Pathological findings of the lymph node (A–G) and skin biopsy (H, I). (A) Haematoxylin-eosin stain, 400. (B) Positive for CD138 (400). (C) Negative for CD20 (400). (D, E) Positive for IgG, and for IgA in a few cells (400). (F, G) Negative for kappa light chain and positive for lambda light chain (400). (H) Haematoxylin-eosin stain, 100. (I) Direct immunofluorescence showed pericellular deposits of IgG (400).

associated with antibody polyreactivity and autoantibody (Fig S6).¹¹ In this context, intraclonal variation and decreased SHM frequency may contribute to the production of various autoantibodies. In addition to our observation, five patients have been reported, who had plasma cell-type PTLD and autoimmune disease (Table SIII).^{12–16} All the patients developed AIHA and/or ITP after SOT. Four patients developed diseases within 16 months, whereas plasma cell-type PTLD usually demonstrates a delayed onset.² These observations suggest that autoreactive plasma cells of recipient origin could contribute to not only PTLD but also autoimmune diseases after transplantation.

In conclusion, we identified the cells of origin and clonal variants in a patient with plasmacytoma-like PTLD, which were markedly different from those in patients with non-PTLD myeloma. The present study provides new insights into the aetiology of PTLD and autoimmune disease after transplantation, and their further understanding will lead to the best interventions for prevention and treatment of these rare but significant complications.

Funding

This work was supported by JSPS KAKENHI Grant Number JP17K17692 to Akihiro Hoshino.

Conflict of interest

The authors have nothing to disclose.

Author contributions

Akihiro Hoshino and Akira Nishimura did conception and design; Akihiro Hoshino, Akira Nishimura, Takuya Naruto, Tsubasa Okano, Hiroshi Shintaku, Shown Tokoro, Hiroyuki Okamoto, Taizo Wada and Keisuke Okamoto performed experiment; Akihiro Hoshino, Akira Nishimura and Kazuaki Matsumoto provided clinical care; Akihiro Hoshino analysed data and wrote the paper; Masatoshi Takagi, Kohsuke Imai, Hirokazu Kanegane and Tomohiro Morio critical revised the paper. All authors reviewed the paper.

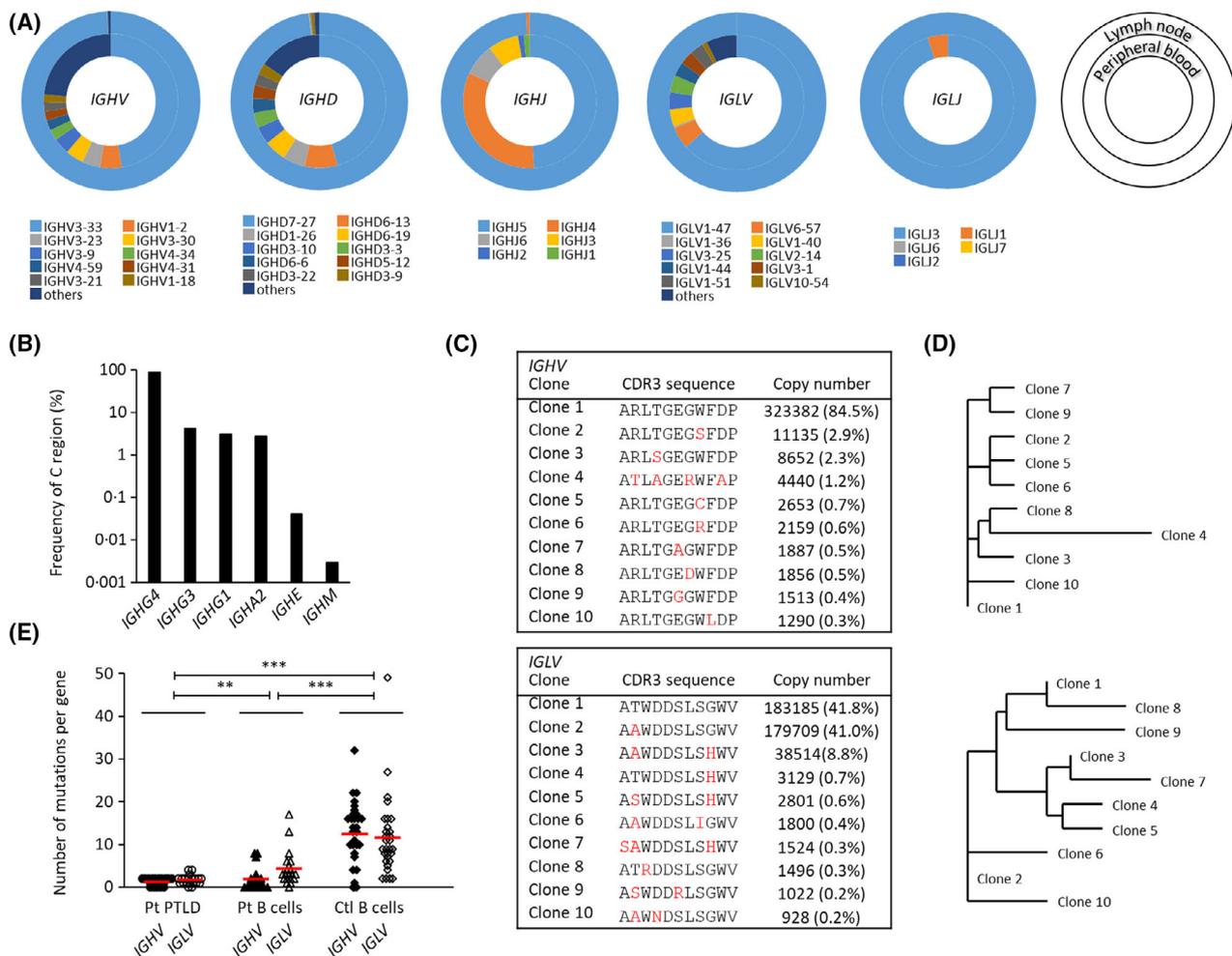


Fig 2. High-throughput sequencing of immunoglobulin heavy- and light-chain genes. (A) V, D and J segment usage of *IGHV/IGL* in lymph node (outer rings) and peripheral blood (inner rings). (B) Constant region usage. (C) Top 10 subclonal sequence variants in *IGHV* and *IGLV*. Red letters indicate the difference in amino acids from clone 1. (D) Phylogenetic tree of the top 10 subclones in *IGHV* and *IGLV*. (E) Frequency of somatic hypermutation in *IGHV* and *IGLV* within patient post-transplant lymphoproliferative disorder cells, patient B cells and control B cells. Red bars indicate means, and *P* values were generated by Student's *t*-test. ** $P < 0.01$. *** $P < 0.001$. CDR3, complementarity-determining region 3; Ctl, control; Pt, patient.

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Keywords: autoimmune disease, plasmacytoma, post-transplant lymphoproliferative disorder, X-linked inhibitor of apoptosis protein (XIAP) deficiency

Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Fig S1. The diagnosis of XIAP deficiency.

Fig S2. Short tandem repeats analysis.

Fig S3. High-throughput sequencing of immunoglobulin kappa light-chain gene.

Fig S4. Hypothetical model of onset of the post-transplant lymphoproliferative disorder and autoimmune diseases.

Fig S5. Flow cytometric analysis of light chain in anti-neutrophil antibodies.

Fig S6. The length of complementarity-determining region 3.

Table SI. Tumour-specific variants.

Table SII. Autoantibodies detected in the patient.

Table SIII. Literature review of plasma cell type post-transplant lymphoproliferative disorder and autoimmune disease.

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