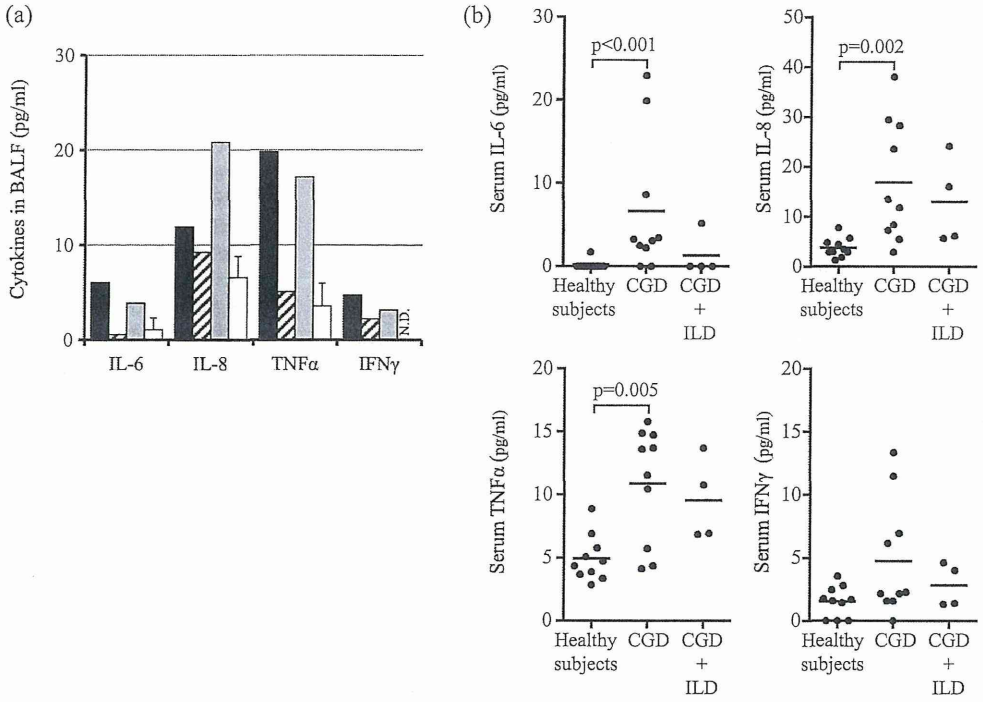

Fig. 5 Cytokine levels in bronchoalveolar lavage fluid and serum are increased in CGD patients. **a** BALF concentrations of IL-6, IL-8, TNFα, and IFNγ for Case 1 (*black bars*), Case 2 (*striped bars*), Case 3 (*gray bars*), and CGD patients who developed pulmonary aspergillosis (white bars, $n=3$). **b** Serum levels of IL-6, IL-8, TNFα, and IFNγ in ILD patients, CGD patients without demonstrable infection ($n=10$), and healthy subjects ($n=10$)

may be insufficient to provide ILD patients with complete therapeutic effects for their clinical symptoms, as their PBMCs lack negative regulators of ROS for inflammatory cytokine production. Meanwhile, anti-inflammatory therapy using steroid (e.g., oral corticosteroid) has been reported to be successful in controlling CGD colitis [25]. However, it should be noted that this therapy often increases a patient's susceptibility to infection [26], as was the situation in Case 1. From this perspective, thalidomide therapy should be considered for CGD patients with ILD because it potentially suppresses inflammation by decreasing inflammatory cytokine production through inhibition of NF-κB. This mode of action has been demonstrated in patients with Behcet's disease, rheumatoid arthritis, and Crohn's disease [27–30]. Also, thalidomide therapy exerts has a smaller negative effect on host defense [31]. Previously, we have reported that thalidomide attenuated excessive inflammation without increasing the susceptibility to infection in a patient with CGD colitis [7].

Conclusions

We described the clinical courses of 4 X-CGD patients with ILD and assessed the functions of PBMCs from CGD patients based on the cells' production of inflammatory cytokines. Although their pathological findings were reminiscent of HP,

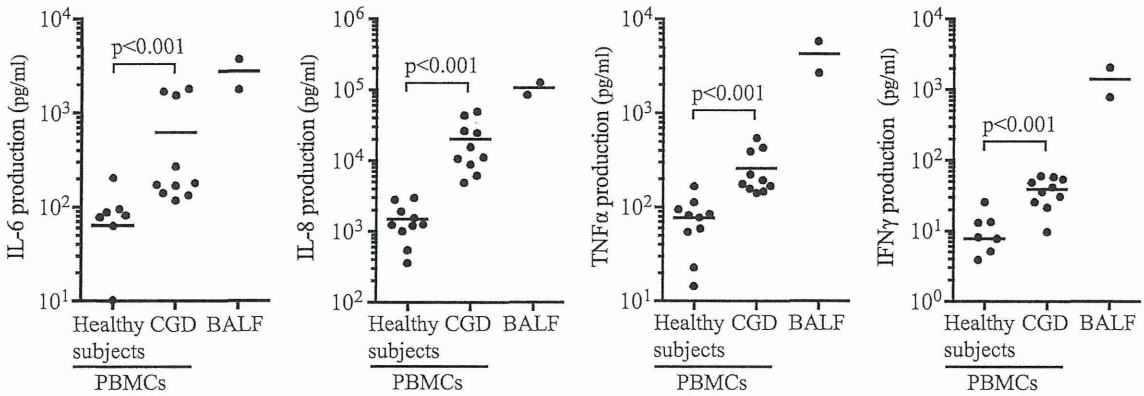

Fig. 6 Cytokine production by PBMCs and infiltrating cells in BALF. Cytokine levels were determined in culture supernatants after PBMCs or BALF cells were cultured for 16 h without stimulation. Concentrations of IL-6, IL-8, TNFα, and IFNγ in culture supernatants of infiltrating cells obtained from BALF and PBMCs obtained from CGD patients without demonstrable infection ($n=10$) and healthy subjects ($n=10$)

it was likely that ILD was caused by excessive inflammation resulting from an inadequate production of ROS in CGD. In addition to CGD-associated bowel inflammation, ILD with microgranulomas may also be one of the characteristic auto-inflammatory diseases for CGD patients.

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Conflict of Interest The authors declare no competing financial interests.

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PALIVIZUMAB USE IN JAPANESE INFANTS AND CHILDREN WITH IMMUNOCOMPROMISED CONDITIONS

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Abstract: A total of 27/28 (96%) immunocompromised Japanese children received ≥4 doses of palivizumab. No respiratory syncytial virus-associated hospitalizations occurred. Mean palivizumab trough concentrations were 59.0 and 91.8 μg/mL 30 days after the 1st and 4th doses, respectively. Of 28 subjects, 27 (96%) experienced ≥1 adverse event and 7 (25%) experienced ≥1 serious adverse event, none of which was considered related to palivizumab.

Key Words: immunocompromised, palivizumab, pediatric, prophylaxis, respiratory syncytial virus

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Respiratory syncytial virus (RSV) is the most common cause of serious respiratory infections in children <5 years. Palivizumab is recommended for the prevention of serious lower respiratory disease caused by RSV in high risk children in a number of countries, including Japan.[1,2] However, children with medical conditions other than those currently approved for palivizumab also are at high risk for serious RSV infections. Congenital or acquired immunodeficiency, immunosuppression after solid-organ or hematopoietic stem cell transplantation or chemotherapy and HIV infection have been shown to be associated with a high risk of serious RSV-associated lower respiratory tract infection.[3] Indeed, immunocompromised conditions have been associated with prolonged hospital stays and higher mortality rates in children with RSV infection compared with children having normal immune function.[3] In addition, the immunologic abnormalities associated with Down syndrome may predispose these individuals to RSV infection.[4–6] A recent survey conducted in Japan determined that a number of hospitalizations and deaths associated with RSV infection occurred in children with immunocompromised conditions.[2]

This study was conducted to evaluate prophylaxis with palivizumab in children ≤24 months of age with immunocompromised conditions and to compare palivizumab trough serum concentrations in these subjects with trough concentrations in patients with medical conditions approved for palivizumab use (infants <6 months born at ≤35 weeks' gestation, pediatric subjects ≤24 months of age with bronchopulmonary dysplasia or pediatric subjects ≤24 months of age with hemodynamically significant congenital heart disease).

MATERIALS AND METHODS

Study Design and Patient Population

This was a multicenter, open-label, uncontrolled, multiple-dose study (ClinicalTrials.gov Identifier: NCT01466062). Eligible subjects were Japanese children ≤24 months of age at the start of study drug administration who had a parent or legal guardian capable and willing to give written informed consent for study participation. The immunocompromising conditions for eligible subjects varied (see Table, Supplemental Digital Content 1, http://links.lww.com/INF/B903). Children who met the approved palivizumab indication in Japan were excluded. Additional exclusion criteria are in Table, Supplemental Digital Content 1, http://links.lww.com/INF/B903. Testing for RSV infection was conducted using a rapid RSV antigen assay for all subjects at screening. Subjects were to receive at least 4 doses of palivizumab (15 mg/kg body weight) by intramuscular injection at approximately 30-day intervals, to a maximum of 7 doses during the RSV season of October 2011 to March 2012.

The study protocol was reviewed and approved by the local independent ethics committee/institutional review board. The study was conducted in accordance with the protocol, International Conference on Harmonisation Guideline for Good Clinical Practice, applicable regulations and guidelines governing clinical study conduct and ethical principles that have their origin in the Declaration of Helsinki.

Outcomes

The primary outcome was the rate of RSV-associated hospitalizations occurring from the initial administration of palivizumab to 30 days after the final dose. RSV testing was conducted in any subject hospitalized with symptoms suspected to be related to RSV infection, or was hospitalized for other reasons between receipt of the initial dose to 30 days after the final dose, and developed symptoms suspected to be related to RSV infection. For secondary outcomes see Table, Supplemental Digital Content 2, http://links.lww.com/INF/B904.

Blood samples were collected at screening, day 31 (before the second dose) and day 121 [30 days after the 4th dose (before the 5th dose)] for determination of serum palivizumab concentrations. Serum palivizumab concentrations were determined by a validated enzyme-linked immunosorbent assay by a central laboratory (data on file). Adverse events (AEs) were assessed and information regarding AE severity, seriousness, time course, duration and outcome and relationship to palivizumab was recorded.

Statistical Analysis

Descriptive statistics were calculated for the full analysis set, defined as all subjects who received at least 1 palivizumab dose.

A 95% confidence interval was calculated for the rate of subjects requiring hospitalization. Descriptive statistics, including mean, median, standard deviation (SD), coefficient of variation and range, were calculated for palivizumab concentrations.

RESULTS

Subject Disposition

A total of 30 subjects were enrolled; 28 received prophylaxis and of those, 26 (92.9%) completed the study. One subject was withdrawn because of an AE and consent was withdrawn for 1 other subject. For subject demographics, see Table, Supplemental Digital Content 3, http://links.lww.com/INF/B905. All subjects were Japanese; most were male (n = 17; 60.7%) and none were born as part of multiple birth pregnancies. The mean age at enrollment was 14.2 months and the mean GA at birth was 38.8 weeks. Rationales for palivizumab prophylaxis are presented in Table 1. The most common reason for prophylaxis was "other immunosuppressive therapy" (n = 11; 39.3%), although 12 subjects met multiple criteria.

Treatment Received

Subjects received a mean (SD) of 6.2 (1.25) palivizumab doses. All 28 subjects receiving prophylaxis received ≥2 doses, 27 subjects (96.4%) received ≥4 doses and 16 subjects (57.1%) received 7 doses.

Hospitalizations and Palivizumab Concentrations

None of the subjects were hospitalized because of an RSV infection (the primary efficacy outcome); thus, the incidence of hospitalization was 0.0% (95% confidence interval: 0.0–12.3). Therefore, no subject required any of the treatments listed as secondary efficacy outcomes. Three subjects experienced a total of 4 respiratory infections suspected to be RSV; however, results of RSV antigen testing for all 4 cases were negative.

Mean (SD) serum palivizumab trough concentrations were 59.0 (12.9) μg/mL at day 31 (30 days after the 1st dose) and increased to 91.8 (40.6) μg/mL at day 121 (30 days after the 4th dose; Table 1). Similarly, when stratified by immunocompromised condition, palivizumab trough concentrations were higher on day 121 than day 31 (Table 1). On day 31, mean (SD) palivizumab trough concentrations varied from 48.0 (6.82) μg/mL (post-organ transplantation) to 69.3 (9.70) μg/mL (Down syndrome). On day 121, mean (SD) palivizumab concentrations varied from 64.9 (30.2) μg/mL (post-organ transplantation) to 122.0 (28.2) μg/mL (high-dose corticosteroid therapy); however, there was considerable overlap in the individual concentration ranges. Overall, the serum palivizumab trough concentrations on day 31 and day 121 appeared comparable across the various groups of patients categorized by immunocompromised conditions.

Adverse Events

Twenty-seven subjects (96.4%) experienced ≥1 AE (see Table, Supplemental Digital Content 4, http://links.lww.com/INF/B906). The AEs that occurred most frequently in ≥10% of subjects were upper respiratory tract infection, gastroenteritis and eczema. Seven subjects (25.0%) experienced ≥1 serious AEs. The most frequently occurring serious AEs were bronchitis (n = 2; 7.1%) and gastroenteritis (n = 3; 10.7%). Most AEs were considered to be mild or moderate, but 2 subjects experienced severe AEs. One subject diagnosed with biliary atresia had undergone organ transplantation and was receiving high-dose corticosteroid therapy and immunosuppressive therapy and experienced encephalopathy that began on day 104 after the 4th dose of palivizumab. The subject subsequently discontinued from study and had not recovered from this AE by the end of the study. Another subject, diagnosed with Down syndrome, experienced gastrointestinal perforation and infectious peritonitis. None of the serious AEs were considered related to palivizumab. There were no deaths during the study.

DISCUSSION

In this study of 28 children with immunocompromised conditions, no RSV-associated hospitalizations occurred after prophylaxis with palivizumab. Although palivizumab trough concentrations vary between subjects, the mean serum palivizumab trough concentrations measured in the subjects in this study were comparable with Western subjects (37–72 μg/mL in children receiving from 1 to 4 palivizumab injections).[7] In addition, mean (SD) palivizumab trough concentrations in Japanese pediatric subjects with immunocompromised conditions were comparable with Japanese pediatric subjects with hemodynamically significant congenital heart disease at 30 days after the 1st [59.0 (12.9) vs. 57.2 (11.7) μg/mL] or 4th doses [91.8 (40.6) vs. 90.2 (23.7) μg/mL; data on file] or Japanese pediatric subjects who were born prematurely or who had bronchopulmonary dysplasia at 30 days after the 1st dose [59.0 (12.9) vs. 50.5 (17.5) μg/mL].[8] Palivizumab was well-tolerated in the population enrolled in this study.

Prior studies demonstrate that palivizumab may be appropriate for use in children who are immunosuppressed. A survey conducted in the United States in 2006 among pediatric solid-organ transplant programs found that approximately 40% of the 67

TABLE 1. Serum Palivizumab Trough Concentrations

Reason for Prophylaxis*	n (%)	Mean ± SD (range), μg/mL	
All subjects	18 (100)	59.0 ± 12.9 (36.6–84.1)	91.8 ± 40.6 (29.8–181)
Immunodeficiency†	4 (14.3)	53.6 ± 16.8 (36.6–76.0)	82.4 ± 38.2 (30.0–119)
Down syndrome without current hemodynamically significant CHD	5 (17.9)	69.3 ± 9.70 (61.0–79.9)	77.5 ± 34.3 (29.8–110)
Organ transplantation	8 (28.6)	48.0 ± 6.82 (37.7–56.3)	64.9 ± 30.2 (37.3–107)
Bone marrow transplantation	4 (14.3)	58.7 ± 17.4 (46.2–84.1)	117 ± 46.2 (81.1–181)
Immunosuppressive chemotherapy	5 (17.9)	61.6 ± 7.95 (51.8–69.8)	107 ± 36.8 (75.3–150)
High-dose corticosteroid therapy‡	6 (21.4)	62.0 ± 8.03 (51.3–74.5)	122 ± 28.2 (78.6–152)
Other immunosuppressive therapy§	11 (39.3)	51.2 ± 9.85 (37.7–74.5)	73.7 ± 30.2 (37.3–119)

*Some subjects had multiple immunocompromised conditions.

†Combined immunodeficiency (eg, severe combined immunodeficiency, X-linked hyper-IgM syndrome), antibody deficiency (eg, X-linked agammaglobulinemia, common variable immunodeficiency, non–X-linked hyper-IgM syndrome) or other immunodeficiency (eg, Wiskott-Aldrich syndrome).

‡Prednisone equivalents ≥0.5 mg/kg every other day, other than inhaler or topical use.

§Azathioprine, methotrexate, mizoribine, mycophenolate mofetil, cyclophosphamide, cyclosporine, tacrolimus and cytokine inhibitors.

CHD, congenital heart disease.

responding programs used palivizumab prophylactically in candidates or recipients of organ transplants.[9] The rate of RSV infection was 4% (4/109) in transplant recipients who received palivizumab prophylactically and 11% (22/195) in transplant recipients who did not receive palivizumab ($P = 0.03$).[9] A Phase 1 study of palivizumab in pediatric and adult patients who received hematopoietic stem cell transplants demonstrated that pharmacokinetic and safety parameters should not preclude palivizumab use in this population.[10]

A strength of this study was the inclusion of subjects who were immunocompromised because of a number of different conditions. A limitation is the relatively low number of subjects per included medical condition. No HIV-infected children were included in large part because of the low incidence in the patient group being studied. It should be noted that palivizumab may interfere with immune-based RSV diagnostic tests, such as some antigen-detection based assays, and could lead to false-negative RSV diagnostic test results.

No significant AEs were identified with prophylactic use of palivizumab in children with immunocompromised conditions. This study also demonstrated similar palivizumab trough concentrations among indicated and immunocompromised Japanese populations, suggesting that immunocompromised children 24 months of age or younger could benefit from prophylaxis with palivizumab during the RSV season.

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CYTOKINE CONCENTRATIONS IN PEDIATRIC PATIENTS WITH CRIMEAN-CONGO HEMORRHAGIC FEVER

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Abstract: Crimean-Congo hemorrhagic fever is a zoonotic disease that can be a severe illness in humans. We investigated concentrations of interleukin (IL)-6, tumor necrosis factor-α and IL-10 in serum samples obtained from 25 pediatric Crimean-Congo hemorrhagic fever cases and 35 control children with no signs of infection. Lower cytokine values in our patients could be a good prognostic factor to for a better outcome.

Key Words: Crimean-Congo hemorrhagic fever, children, cytokine, mortality

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Crimean-Congo hemorrhagic fever (CCHF) is a potentially fatal viral infection found in parts of Africa, Eastern Europe and the Middle East.[1] The CCHF virus belongs to the genus *Nairovirus* in the family of Bunyaviridae and causes a severe disease in humans, with a reported mortality rate of 3–30 %.[2–4] Large scale outbreaks have also taken place in the middle, northern and eastern regions of Turkey during spring and summer seasons since 2002.[4–7] The case fatality rate (CFR) reported from The Ministry of Health of Turkey is 5%.[6] Although adults constitute the major group of impact by CCHF, children are also affected by the disease.[8] According to unpublished data of The Ministry of Health of Turkey between 2008 and 2012, CFR was 1.9% in children aged between 0 and 18 years of age. These data suggest that CCHF in children has a milder course than in adults.[8–12] Although the pathogenesis of CCHF has not been well-defined, inflammatory processes and release of proinflamatory cytokines such as interleukin (IL)-1, IL-6 and tumor necrosis factor (TNF)-α were reported to be important factors in disease pathogenesis and disease course in viral hemorrhagic fever.[13–15] In this study, the relationship between cytokine values and the course of CCHF in pediatric patients is evaluated.

STUDY DESIGN

The study included 25 patients <18 years of age admitted to the Ankara Hematology Oncology Children's Training and Research Hospital between January 2010, and November 2013,

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Basiliximab treatment for steroid-resistant rejection in pediatric patients following liver transplantation for acute liver failure

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Abstract: An IL-2 receptor antagonist, basiliximab, decreases the frequency of ACR in liver transplant (LT) recipients as induction therapy. The aim of this study was to evaluate the effectiveness of basiliximab against SRR as rescue therapy in pediatric LT patients with ALF. Forty pediatric ALF patients underwent LT between November 2005 and July 2013. Among them, seven patients suffering from SRR were enrolled in this study. The median age at LT was 10 months (6–12 months). SRR was defined as the occurrence of refractory rejection after more than two courses of steroid pulse therapy. Basiliximab was administered to all patients. The withdrawal of steroids without deterioration of the liver function was achieved in six patients treated with basiliximab therapy without patient mortality, although one patient developed graft loss and required retransplantation for veno-occlusive disease. The pathological examinations of liver biopsies in the patients suffering from SRR revealed severe centrilobular injuries, particularly fibrosis within one month after LT. We demonstrated the effectiveness and safety of rescue therapy consisting of basiliximab for SRR in pediatric LT recipients with ALF.

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Calcineurin inhibitors, such as TAC and cyclosporine, combined with steroids are standard ISs, and their use has reduced the frequency of ACR following LT (1). ACR frequently occurs in pediatric LT patients, at a previously reported rate of 41–55% (2, 3). Steroid therapy remains the mainstay of the initial management of acute rejection, coupled with an increase in baseline immunosuppression (4). Although steroid pulse therapy is the first therapeutic option for treating ACR, SRR occurs in 8.1–34% of LT recipients, which results in graft and patient loss (3, 5, 6). The administration of additional ISs, such as MMF, SRL, and antithymocyte globulin, has previously been reported as rescue therapy for SRR (7–9). The efficacy of antithymocyte globulin in treating SRR is largely attributed to its ability to deplete T cells (4). The successful use of antilymphocyte therapy for pediatric patients suffering from SRR and late ACR with cholestasis was reported (10). Although antithymocyte globulin is effective for SRR, sepsis remains a significant complication (7). While SRR was previously an immediate indication for potent antilymphocyte preparations, this is now effectively treated with chimeric or humanized IL-2 receptor monoclonal antibodies (4).

Basiliximab (Simulect™; Novartis Pharma, Basel, Switzerland), a chimeric monoclonal antibody that blocks the α-subunit (CD25) of the IL-2 receptor in association with activated T

Abbreviations: ACR, acute cellular rejection; ALF, acute liver failure; ALT, alanine aminotransferase; AST, asparate aminotransferase; BW, body weight; CMV, cytomegalovirus; EBV, Epstein–Barr virus; IL, interleukin; IS, immunosuppressant; LDLT, living donor liver transplantation; LT, liver transplantation; MMF, mycophenolate mofetil; mPSL, methylprednisolone; PCP, *Pneumocystis* pneumonia; POD, postoperative day; RAI, rejection activity index; SRL, sirolimus; SRR, steroid-resistant rejection; TAC, tacrolimus; Treg, $CD4^+$ $CD25^{high}$ $CD127^{low/neg}$ regulatory T.

helper cells, is usually used as induction therapy in LT (11). The use of basiliximab contributes to reducing the incidence of ACR and allows the dose of calcineurin inhibitors and corticosteroids to be decreased following pediatric LT, with a low incidence of side effects (4, 12–14). However, basiliximab did not affect the long-term patient or graft survival after LT (15). Few studies have reported the use of basiliximab for SRR as rescue therapy (16, 17).

ALF is a rare and fatal disease with a high mortality rate in children (18). The outcomes of LT in patients with cryptogenic ALF are poor in comparison with patients with cholestatic disease, particularly in infants (19–22). The outcomes of pediatric LT for ALF have improved recently, and the patient and graft survival rates at one yr were 71–93.4% and 64–88%, respectively (23–26). The main cause of death was reported to be sepsis, and the incidence of ACR was 36.7–39.4%. On the other hand, certain data also suggest that the patient survival rate among infants with ALF following LT was only 40% at one yr due to the high incidence of SRR (19–22). It has been reported that SRR accounts for 50% of the deaths in children who have undergone LT for ALF. Moreover, it has been speculated that the long-lasting unknown origin of ALF may cause an accelerated immune response in children even after successful LT (19). Therefore, reducing the frequency of SRR is key for improving the short- and long-term survival among pediatric patients with ALF.

The aim of this study was to evaluate the effectiveness of basiliximab against SRR as rescue therapy in pediatric patients with ALF who have undergone LT.

Patients and methods

Forty pediatric ALF patients underwent LT between November 2005 and July 2013 at the National Center for Child Health and Development. During the same period, 195 pediatric patients with non-ALF liver diseases underwent LT. The original diseases in the non-ALF group consisted of biliary atresia in 106, metabolic disease in 45, congenital hepatic fibrosis/Caroli disease in 14, and other diseases in 30 cases. The median age at LT in the ALF group and non-ALF group was 10 months (17 days–12 yr four months) and 16 months (two months–17 yr 11 months) and the median BW was 8.2 kg (2.6–32 kg) and 9.4 kg (3.7–63.8 kg), respectively. Among them, seven patients in ALF group who were diagnosed with SRR were enrolled in this study.

Basic immunosuppression protocol at our hospital and the diagnosis of ACR

Basic immunosuppressive treatment after LT consisted of TAC and low-dose steroids. Briefly, the trough level of TAC was maintained between 10 and 12 ng/mL for the first month and between 8 and 10 ng/mL for the subsequent three months. mPSL was administered at a dose of 1 mg/kg from POD 1–3, 0.5 mg/kg from POD 4–6 and 0.3 mg/kg on POD 7. Prednisolone was given orally at a dose of 0.3 mg/kg from POD 8–28 and 0.1 mg/kg after POD 29. In the patients with ALF, which has previously been reported to be associated with a higher incidence of ACR, the treatment with prednisolone was continued for longer than six months after LT until the liver function became stable (20).

The indication for a liver biopsy was based on the presence of increased AST (normal range: 24–50 IU/L) and ALT (normal range: 9–34 IU/L) levels more than three times the upper limit of the normal range or an increase of more than 50% over the previous record. The histological diagnosis and grading of ACR were determined according to the Banff schema (27, 28). The grade of centrilobular fibrosis was classified as follows: F0: absence of fibrosis, F1: fibrous central vein expansion, F2: central vein fibrosis with incomplete septa, F3: C-C bridging fibrosis, and F4: cirrhosis (29). The presence of veno-occlusive disease was assessed by liver biopsy in all patients. The patency of hepatic vessels, including outflow blockage of the hepatic vein, was routinely investigated after LT using Doppler ultrasonography.

Management of ACR

All rejection episodes were treated with a color steroid bolus injection, generally given at a dose of 10 mg/kg of mPSL for three days. SRR was defined as the occurrence of refractory rejection after two cycles of steroid pulse therapy. The use of basiliximab and antithymoglobulin for liver transplant patients is not still covered by the universal health insurance system in Japan because they are currently unauthorized drugs. In this study, basiliximab treatment was indicated for patients suffering from SRR, because infections remain a significant complication of antithymoglobulin therapy (7). Basiliximab, which was administered at a dose of 10 mg twice on days 0 and 4, was adopted as rescue therapy in the SRR patients.

Characteristics of the SRR group before basiliximab therapy

Characteristics of the SRR group are presented in Table 1. The age and BW in SRR group were 6–12 months (median 10 months) and 7.6–9.6 kg (median 8 kg), respectively. Multiple cycles of steroid pulse therapy, ranging from two to 17 cycles, were performed. Cases 3 and 4 underwent more than 10 cycles of steroid pulse therapy because they frequently suffered from ACR before the introduction of basiliximab therapy for SRR at our hospital. Additional ISs, such as MMF and SRL, were indicated in five patients (MMF in two, SRL in one, and both of them in two patients). Basiliximab was administered beginning 18–762 days after LT. A second dose of basiliximab was given to all but three patients, who did not receive the subsequent dose due to CMV infection (Cases 1 and 6) and graft failure (Case 5).

Management of opportunistic infections after LT

The treatment for CMV infection was preemptively performed at our institute, which was described elsewhere (30). Briefly, CMV-pp65 antigenemia was monitored weekly for

Table 1. Characteristics of the recipients with SRR

Case	Age at LT	No. of steroid pulse treatments	IS used for the treatment of SRR	Age at basiliximab therapy	Timing of basiliximab after LT	Dose of basiliximab
1	6 months	3	TAC, mPSL	2 yr 2 months	POD 44	10 mg
2	7 months	3	TAC, mPSL, SRL	2 yr 10 months	POD 29/38	10 mg/10 mg
3	9 months	12	TAC, mPSL, MMF, SRL	2 yr 2 m	POD 762/771	10 mg/10 mg
4	10 months	17	TAC, mPSL, MMF, SRL	2 yr 10 months	POD 524/528	10 mg/10 mg
5	10 months	5	TAC, mPSL, MMF	1 yr 7 months	POD 262	10 mg
6	10 months	3	TAC, mPSL	10 months	POD 18	10 mg
7	12 months	2	TAC, mPSL, MMF	12 months	POD 42, 46	10 mg/10 mg

the first three months after LDLT, during treatment for ACR or when the patients presented with symptoms and laboratory data suspected to indicate CMV infection. If the presence of more than five CMV antigen-positive cells/ 50 000 white blood cells was revealed, intravenous ganciclovir (5 mg/kg/dose, every 12 h) was initiated for the first two wk, followed by a maintenance dose of intravenous ganciclovir (5 mg/kg/dose, every 24 h) until the CMV-pp65 antigenemia became negative.

The EBV management protocol after LDLT at our institute was described elsewhere (31). Briefly, the EBV viral loads in the peripheral blood were detected using a real-time quantitative polymerase chain reaction method and were monitored once per week for the first two months after LDLT, followed by every 1–3 months. If the symptoms and laboratory data suggested EBV infection or there were high values of EBV-PCR (more than 10^2 copies/μg DNA), immunosuppression was withdrawn.

Preventive therapy for PCP was administered according to the prophylactic use of trimethoprim–sulfamethoxazole (0.05 mg/kg/day) for the first three months after LDLT.

Monitoring of the $CD4^+$ $CD25^+$ T cells and regulatory T calls before and after basiliximab therapy

Monitoring of peripheral $CD4^+$ $CD25^+$ T cells and Treg cells was performed in the patients receiving basiliximab therapy for SRR (n = 6, Cases 1, 2, 4–7). Peripheral blood samples obtained from the patients and healthy controls were subject to Ficoll–Hypaque density gradient centrifugation to isolate the peripheral blood mononuclear cells. Peripheral blood lymphocyte subsets were determined with a FACSAria IIIu instrument (Becton Dickinson, Mountain View, CA, USA) using anti-human CD4, CD127, and CD25 monoclonal antibodies conjugated with fluorescein isothiocyanate, phycoerythrin, or allophycocyanin (BioLegend, San Diego, CA, USA), respectively. The FlowJo software program (TreeStar Inc., Ashland, OR, USA) was used for all aspects of the data analyses. The levels of $CD4^+$ $CD25^+$ T cells were monitored to assess the effects of basiliximab, and the levels of Treg cells were monitored because human Treg cells have been demonstrated to have great potential for use in therapeutic interventions to prevent graft rejection (32). Blood samples were obtained before and one wk after the administration of basiliximab therapy. The data of healthy children of similar age were collected as healthy controls (n = 7; male: 4, female: 3, five months–three yr three months, median: one yr seven months). Informed consent was obtained from all patients, healthy children, and their parents.

Statistic analysis

The statistical analysis was performed using the Kruskal–Wallis test for the analysis of the median age and BW, and the Tukey test for the analysis of the flow cytometry data. Proportions were compared using the chi-square test. p-Values of <0.05 were considered to be significant. Patient survival and graft survival were evaluated according to the Kaplan–Meier method and compared using the log-rank test. The software program SPSS version 18.0 (SPSS, Inc., Chicago, IL, USA) was used for the statistical analysis.

Results

Comparison of the characteristics of the ALF group and non-ALF group

Biopsy-proven ACR developed in 24 (60%) and 75 patients (38.5%), respectively, in the ALF and non-ALF groups (p = 0.014). Moreover, SRR was revealed in seven (17.5%) and five patients (2.6%) in the ALF group and non-ALF group, respectively (p = 0.001). The overall survival of the 40 patients with ALF at one and five yr was lower than that of the non-ALF group (84.8% and 84.8% vs. 92.1% and 91.4%, respectively), but the differences were not significant (p = 0.23).

Comparison of the characteristics of the non-ALF group, ALF group, and SRR group among the ALF patients

The median age at LT in the non-ACR group was higher than that in the other group, but the difference was not significant (p = 0.427). All of the cases in the ACR and SRR groups received grafts from live donors rather than deceased donors (p = 0.036). The etiology of ALF was unknown in all patients in the SRR group (p = 0.215) (Table 2).

The outcomes of ALF patients suffering from SRR

No patients have died as a result of SRR or chronic rejection thus far. The basiliximab treatment outcomes for SRR are shown in Table 3.

Table 2. The demographics of the non-ACR, ACR, and SRR groups of pediatric ALF patients after LT

	non-ACR	ACR	SRR	p
n	16	17	7	
Age at LT (median)	17 days–12 yr 4 months (1 yr 3 months)	1 months–4 yr 4 months (9 months)	6 months–12 months (10 months)	0.427
BW at LT (kg [median])	2.6–32 (8.1)	3.5–17 (8.6)	7.6–9.6 (8)	0.669
Gender male/female	8/8	12/5	5/2	0.411
Living donor (n [%])	13 (76.5)	17 (100)	7 (100)	0.036
ABO-incompatible (n [%])	4 (25)	5 (29.4)	1 (14.3)	0.739
Etiologies (n [%])				
Unknown	11 (68.8)	14 (82.4)	7 (100)	0.215
Others	5 (31.3)	3 (17.7)	0 (0)	
EBV	2 (12.5)	1 (5.9)		
CMV	1 (6.3)			
HSV 1		1 (5.9)		
Echovirus 3		1 (5.9)		
MDS	1 (6.3)			
Hemochromatosis	1 (6.3)			

HSV, herpes simplex virus; MDS, mitochondria DNA depletion syndrome.

Table 3. The outcomes of basiliximab therapy in the SRR patients

Case	The interval between treatment and discharge (days)	ACR after basiliximab	CMV	EBV	Other infection	Outcome	Follow-up (months)	Present IS
1	18	+	+	–		Alive	27	TAC
2	90	+	+	–	Pneumocystis pneumonia	Alive	24	PSL, SRL
3	40	–	+	–		Alive	47	TAC
4	23	–	+	–		Alive	51	TAC
5	223	+	+	+		Graft loss, retransplant (POM 9), Alive	18	TAC, PSL, MMF, SRL
6	41	–	+	+		Alive	15	TAC, PSL
7	23	–	+	+		Alive	2	TAC, PSL, MMF

POM, postoperative months.

All patients survived, although one patient (Case 5) required retransplantation due to graft failure. The histopathological findings of his explanted graft revealed severe centrilobular rejection, including severe necrosis of zone 3, accompanied by suspected veno-occlusive disease. Veno-occlusive disease was not present in the other patients. Three patients (Cases 1, 3, and 4) could discontinue steroids after basiliximab therapy. Two patients (Cases 2 and 6) remained on prednisolone for more than two yr and one yr after basiliximab therapy, respectively. Two patients required low doses of prednisolone due to a slight increase of the values of liver function tests after basiliximab therapy at the end of the follow-up.

The changes in the results of liver function tests, such as the levels of total bilirubin, AST, and ALT after basiliximab therapy in all but one patient (Case 5) are shown in Fig. 1, which revealed a dramatic improvement of the liver function test after basiliximab therapy. The patients were discharged from the hospital with a median period from the date of basiliximab therapy to the resolution of ACR of 31.5 days (range: 18–90 days). In spite of improvement of the liver function, the duration of hospitalization was extended because the administration of ganciclovir was required in all but one patient (Case 5). Three patients (Cases 1, 2, and 5) experienced ACR at seven, 10, and 1.5 months after basiliximab therapy, respectively. All patients improved following treatment with single steroid pulse therapy. There was the same sentence in this paper.

Infectious complications after basiliximab therapy

Opportunistic infections were common, as evidenced by the CMV and EBV viremia and pneumocystis pneumonia. None of the patients developed CMV disease, although CMV-pp65 antigenemia was detected in all patients after basiliximab therapy. CMV immunoglobulin-G was

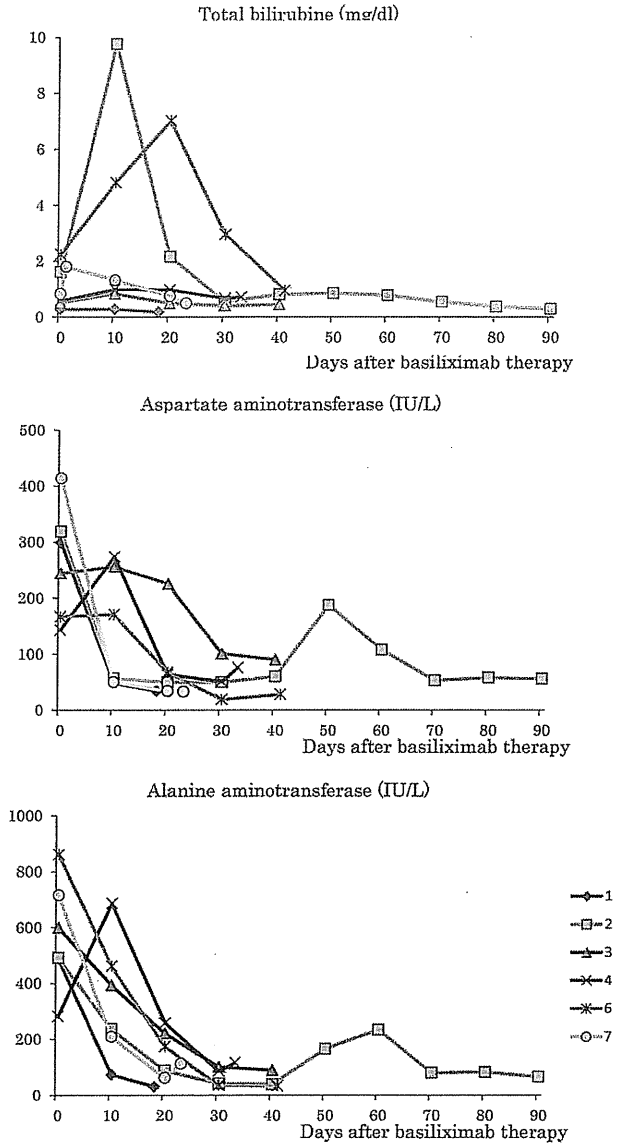

Fig. 1. Changes in liver function test parameters in six patients who exhibited improvements from the administration of basiliximab therapy to hospital discharge.

positive in all recipients before LT, and all but one (Case 5) had received livers from CMV-positive donors. The CMV-pp65 antigenemia in Cases 1 and 6 became positive within four days after basiliximab treatment; thus, the second dose of basiliximab was not administered. The CMV-pp65 antigenemia in Cases 2, 3, 4, and 7 became positive within two wk after basiliximab therapy, and in Case 5, it became positive after retransplantation. Although EBV-DNA was positive in three patients (Cases 5, 6, and 7) during the follow-up period, none of the patients developed post-transplant lymphoproliferative disease. One patient (Case 2) suffered from PCP nine months after basiliximab therapy and was successfully treated with the intravenous administration of trimethoprim–sulfamethoxazole.

The histopathological findings of the SRR patients

The histopathological findings are shown in Table 4. The histopathological grade of rejection on the Banff criteria was classified as moderate to severe rejection in all patients, and the venous endothelial inflammation scores for the central vein region were high. Centrilobular fibrosis was observed in six patients at the first rejection episode, with an interval from 11 to 44 days after LDLT. Centrilobular necrosis and hemorrhage were detected in the majority of patients. The RAI, including the presence of centrilobular perivenulitis, improved in three patients who received follow-up biopsies. No patient showed an anastomotic stricture of the hepatic vein by Doppler ultrasonography.

The changes in the proportions of $CD4^+CD25^+$ T cells and regulatory T cells before and after basiliximab therapy

The changes in the proportions of $CD4^+CD25^+$ T cells and Treg cells in the peripheral blood before and after basiliximab therapy are shown in Fig. 2. The proportions of $CD4^+CD25^+$ T cells in the patients before basiliximab therapy (mean ± standard deviation: 16.56 ± 6.49%) were significantly higher than those observed in the healthy control children (9.67 ± 2.17%, $p = 0.034$). The proportions of $CD4^+CD25^+$ T cells were significantly suppressed after basiliximab therapy compared to those measured in the patients before basiliximab therapy (16.56 ± 6.49% vs. 9.07 ± 3.96%, $p = 0.026$). There were no significant differences in the proportion of Treg cells in the peripheral blood measured before basiliximab therapy in the patients and those observed in the healthy control children (6.18 ± 3.01% vs. 6.91 ± 2.04%, $p = 0.853$). The proportions of Treg cells were also suppressed in the patients after basiliximab therapy compared to those observed in the patients before basiliximab therapy (2.71 ± 2.21% vs. 6.18 ± 3.01%, $p = 0.062$). The proportions of $CD4^+CD25^+$ T cells and Treg cells in the patients who developed graft loss after basiliximab therapy were 3.13% and 1.52%, respectively, which were the same as those observed in the other patients.

Discussion

Previous studies of LDLT for ALF in children, especially infants, have documented poor outcomes. Possible reasons for these results include