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A shared regulatory perspective on deferral from blood donation of men who have sex with men (MSM)†

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National Regulatory Authorities (NRAs) establish deferral criteria for donors with risk factors for transfusion transmissible infections (TTI). In most jurisdictions, epidemiological data show that men who have sex with men (MSM) have a significantly higher rate of TTI than the general population. Nevertheless, changes from an indefinite donor deferral for MSM have been considered in many countries in response to concerns over a perceived discrimination and questioning of the scientific need. Changes to MSM donor deferral criteria should be based on sound scientific evidence. Safety of transfusion recipients should be the first priority, and stakeholder input should be sought.

Key words: blood collection, donors, epidemiology, motivation/recruitment, transfusion medicine (in general).

Background

To protect patients receiving blood components against exposure to transfusion transmissible infectious agents (TTI), National Regulatory Authorities (NRAs) require blood collection establishments to screen blood donors through a combination of a health questionnaire and laboratory testing procedures. Available laboratory tests are highly sensitive for detection of the major TTI. However, testing is not available for all significant TTI; laboratory tests cannot reliably detect donor infections with TTI during the early infection period; and procedural errors are possible [1–4]. Therefore, deferral from donation of persons with increased risk of contracting TTI takes on importance as a key safety measure to protect transfusion recipients against TTI.

To be effective as a safety measure, the health questionnaire helps to identify risk factors, including certain sexual behaviours, whose association with TTI is established through epidemiological studies. In particular, male sex with other males has been associated with increased risk of TTI [5–9]. However, policies to defer blood donors indefinitely based on a history of male sex with another male have been controversial in many countries due to perceptions of discrimination. Questions also have been raised whether such a deferral is necessary or effective [10–17]. In principle, longer durations of deferral for MSM may not inherently result in lower risks of TTI in

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donors unless those criteria select for donors at lower risk of recent infection. Lower TTI risk was correlated with an MSM abstinence period of longer than 5 years in one study [18].

National Regulatory Authorities that establish risk-based donor deferral criteria have emphasized that these policies attempt to exclude potential donors with identified risk factors. In the case of MSM, the donor deferral is based on the risk that is associated with the behaviour (i.e. sexual behaviours that constitute an elevated risk to acquire TTI) and is not based on the sexual orientation per se of the donor. The consequent application of this criterion is of high importance for the safety of the blood supply and is not meant to discriminate against any individual or group. In most jurisdictions, epidemiological and public health data have shown and continue to show that men who have had sex with other men have a significantly higher rate than the general population of acquiring, carrying and transmitting certain infectious agents, such as HIV, hepatitis and potentially other TTI [5–9].

Because of the general difficulty in assessing individual risk related to sexual behaviour [19], many NRAs have chosen to apply a uniform MSM deferral. Similar considerations regarding assessing individual risk have been applied to exclude donors with a history of illicit injection drug use, sex workers and donors with exposure in certain geographical areas where risk of acquiring TTI is increased.

Current situation

Similar epidemiological data showing increased TTI risk among MSM exist in various jurisdictions, and many NRAs worldwide have a policy of indefinite deferral for MSM. Although there are variable results, many modelling studies have shown that in changing the deferral period from indefinite to 1, 5 or 10 years, a very small absolute increase in risk cannot be ruled out [17, 18, 20–22]. In a recent retrospective analysis in Australia, the HIV prevalence of donors was found not to have increased following a change to a time-based 12-month deferral [23]. A number of countries have changed the MSM deferral from an indefinite to a time-based deferral. Among these countries are Australia, Canada and Japan who have contributed to this publication and share the regulatory perspective described herein. Some other countries have replaced a uniform deferral for MSM behaviour with donor questioning to identify recent high-risk sexual exposure (e.g. Italy, Mexico, Poland, Russia, Spain).

The available scientific data and the policies regarding deferral on the basis of sexual behaviour in different jurisdictions have recently been analysed [24, 25]. In some countries, studies have been undertaken to better understand the attitudes and behaviour of MSM towards donation, the adequacy of the donor history questionnaire to identify donors at increased risk of TTI, and the impact of potential or actual changes from a policy of indefinite deferral for MSM. Similar to the conclusion of another report [9], the authors noted that overall risk to transfusion safety would be influenced to a large extent by the degree of adherence by MSM to revised deferral criteria [23, 26].

A less restrictive policy on blood donation by MSM has been projected to produce only a small public health benefit through an increase in blood donations [16]. Any projected increase to the blood supply has not been thought by many NRAs to be sufficient to warrant a policy change at this time. Many NRAs have taken the view that any preventable projected increase in risk to the blood supply from MSM donation should not be imposed on recipients of blood components in the absence of a countervailing benefit to recipients.

Considerations for decision-makers

Scientific evidence

Decision-making for NRAs is predicated on relative benefit to risk considerations informed by current scientific understanding and available evidence. Factors that may be relevant when considering changes from an indefinite MSM deferral could be as follows:

(1) Evidence from other jurisdictions which have changed the deferral from indefinite to a time-based deferral indicating that there has been no increase in risk (e.g. based on TTI marker rates, especially window period infections, prevalence in first time donors, and rates of seroconversion in repeat donors) and no observed increase in the frequency of pathogen transmissions based on sound haemovigilance monitoring.
(2) Evidence that the current deferral policy is not effective at reducing risk of TTI. In this context, the effectiveness of health questionnaires should be assessed and options for improvements should be explored.
(3) Evidence from well-designed studies that revised donor selection criteria allowing blood donations by some men with a prior history of sex with another man would not be likely to increase the risk of TTI.
(4) Consideration of risk mitigating steps that could be implemented in conjunction with a change to MSM deferral criteria so as to improve current blood safety controls (e.g. predonation testing, quarantine hold pending postdonation testing for some products, single unit nucleic acid testing and pathogen reduction).

(5) Evidence that changes in deferral criteria will not increase risk from inadvertent release of infectious units from quarantine (quarantine release errors).

Societal considerations

National Regulatory Authorities function within a broader framework of society wherein social attitudes, perceptions and priorities are relevant to and may influence policy decision-making. NRAs and other relevant government bodies should seek to receive comments from all stakeholders, when contemplating a change to the MSM deferral policy. The overriding consideration should be that a change in policy should not increase risk to transfusion recipients. However, de minimus changes in risk may be acceptable in the context of larger societal benefits. Social factors affecting the overall safety and adequacy of the blood supply such as compliance with deferral criteria and public willingness to donate blood may be part of the assessment of risks and benefits of a policy change. The discussion should include whether the public is prepared to accept some added risk of transfusion for a possible benefit of reducing a perceived discrimination against MSM.

Conclusion and recommendations

Based on the principle that the safety of blood transfusion for recipients should be the primary concern of the NRA, the authors recommend careful deliberation in considering a less restrictive blood donor deferral for MSM.

Donor deferrals based on sexual behaviour play an important role in preventing disease transmission from TTI. However, despite the clear association of TTI risk with a history of male sex with other males, policies to indefinitely defer MSM from blood donation as a blood safety measure are controversial in many jurisdictions both on scientific and societal grounds. Consequently, many NRAs are now reviewing their current policy of MSM deferral, some have implemented, and others are considering a modification of their existing policy. Time-limited deferrals have been implemented by some NRAs, at least partly in consideration of country-specific socio-political concerns including alleviation of a perceived discrimination and possibly based on tolerance of a potential small increase of risk.

Modelling studies suggest that changes from indefinite to time-limited deferrals will increase TTI risk unless accompanied by an increase in adherence to the deferral criteria by MSM. In particular, the motivations of MSM to donate and the likelihood that a modification of the donor criteria could change the degree of MSM compliance need to be assessed. Implementation of revised deferral criteria for MSM in Australia did not result in an increase in the rate of HIV-positive donations [23]. A more recent study in the same country was interpreted to confirm the previous finding of a high degree of compliance by MSM with a 12-month deferral period [26]. Whether the same outcome could be achieved in different settings cannot be predicted due to differences in epidemiology of infections and societal norms. Improvements in the effectiveness of health questionnaires may contribute to blood safety in conjunction with a policy change on deferral for history of MSM.

The authors believe that changes to the donor deferral for history of MSM should be evidence driven and based on sound science. Consequently, the authors recommend that NRAs consider the following points as part of the overall decision process:

(1) Safety of transfusion recipients should be the first priority of the NRA.
(2) Appropriate studies should be conducted to assess the potential impacts of policy changes, to validate candidate risk mitigation strategies implemented in association with policy changes and to monitor the safety outcomes of a policy change.
(3) Stakeholder input should be sought through a formal process designed to enable all viewpoints to be considered constructively by the NRA.
(4) In order to inform future decision-making, also relating to MSM deferral policy, individual jurisdictions should continuously assess haemovigilance data regarding the risk of TTI in donors. Emphasis should be given to monitoring adherence to donor selection criteria, especially following changes in donor questionnaires or deferral periods.

In conclusion, the authors believe that NRAs should follow the same principles in addressing the issue of donor deferral for MSM, namely priority for patient safety, evaluation of scientific data, consideration of the local epidemiology and societal circumstances, as well as stakeholder input, which may be quite different between countries. However, the authors recognize that based on such differences in the various jurisdictions, decisions based on the criteria stated above may result in different outcomes [22, 26, 27].

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A retrospective observational study to assess adverse transfusion reactions of patients with and without prior transfusion history

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Background and Objectives This study compares the frequency of adverse transfusion reactions (ATRs) after first transfusions with the frequency of ATRs for subsequent (non-first) transfusions.

Materials and Methods Five hospitals agreed to systematically collect and share 2 years of data. This was a retrospective observational analysis of data including the number of transfusion episodes and ATRs for red blood cells (RBCs), fresh frozen plasma (FFP) and platelet concentrates (PCs) given to first-time transfusion recipients and to those previously transfused.

Results First transfusion ATRs to RBCs, FFP and PCs were 1·08%, 2·84% and 3·34%, respectively. These are higher than ATR incidences to RBCs (0·69%), FFP (1·91%) and PCs (2·75%) on subsequent transfusions. Specifically, first transfusion incidences of febrile non-haemolytic transfusion reactions (FNHTRs) to RBCs (0·43%) and allergic reactions to FFP (2·51%) were higher than on subsequent transfusions (RBCs: 0·23%, FFP: 1·65%).

Conclusion There are risks of ATRs on the first transfusion as well as transfusions of patients with transfusion history.

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Key words: adverse transfusion reaction, allergic reaction, first transfusion, haemovigilance, transfusion episode.

Introduction

Although blood transfusion is regarded as an essential and life-saving therapy, significant clinical risks are attributed to the allogeneic origin of blood components. Also, concerns have been expressed about adverse transfusion reactions (ATRs) attributed to cytokines that accumulate in stored autologous blood. As transfusion-transmitted infections decrease due to improved donor screening and blood testing [1], non-infectious ATRs attract greater concern. In fact, the risk of transfusion-transmitted infectious diseases has decreased approximately 10 000-fold [2], while deaths attributed to transfusion-related acute lung injury (TRALI) and haemolytic transfusion reactions now account for approximately 72% of all transfusion-related fatalities reported to the Center for Biologics Evaluation and Research (CBER) of the US Food and Drug Administration [3]. Although blood components in Japan – as elsewhere – can be considered as highly safe, transfusion-associated ATRs have not been eliminated. Indeed, the overall incidence of ATRs per transfused unit was 1·53% in Japan [4]. But, the incidences of ATRs per unique patient per year are still high: to red blood cells (RBCs), 2·61%; to fresh frozen

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plasma (FFP), 4·32%; and to platelet concentrates (PCs), 13·24% [4].

While the majority of ATRs are not serious, these reactions can lead to hospitalization for outpatients or prolonged hospital stays for inpatients. This is a burden to patients, their families and to healthcare under any system of delivery and reimbursement. For these reasons, preventive strategies should be embraced. To this end, it is prudent to quantify ATRs, search for causality and investigate mechanisms.

National haemovigilance systems began in France in 1994 [5] and in the United Kingdom in 1996 [6], although these two systems differ greatly. With the advent of Directive 2002/98/EC [7], the introduction of haemovigilance systems has become a priority throughout the European Community. The recently established US Biovigilance Network commenced data collection in 2008 [8]. Less well known, the Japanese Red Cross Society (JRCS), as a monopoly provider of allogeneic blood, recognized its duty to co-ordinate blood safety and established a haemovigilance system in 1993. In summary, the emergence of haemovigilance systems is a global phenomenon.

Although most of these systems report the incidence of ATRs on a per-transfused unit basis, the incidence of reactions from first vs. subsequent transfusions has not been published, even though some ATRs are associated with prior transfusion. Previous exposure to allogeneic blood may sensitize patients to foreign plasma proteins [9] as well as cell surface alloantigens. Even so, the impact of prior transfusion on subsequent ATRs is not well defined. Herein we report the incidence of first transfusion ATRs vs. subsequent (non-first) transfusion ATRs from five hospitals over 2 years and consider the factors that may account for any differences.

Patients and methods

Study set-up

Data from standardized records were collected by the Department of Transfusion Medicine, Aichi Medical University, from its own and four hospitals. The data covered January 2010 through December 2011 and were assembled in February 2012.

Study design

This was a retrospective observational analysis of data from standardized records in 5 Japanese hospitals with established haemovigilance systems (Aichi Medical University Hospital, Keio University Hospital, Osaka University Hospital, Shinshu University Hospital, and Tokyo Metropolitan Cancer and Infectious Diseases Center Komagome Hospital) from January 2010 through December 2011, covering transfusions and associated ATRs for red blood cells (RBCs), fresh frozen plasma (FFP) and platelet concentrates (PCs). The study was approved by the Aichi Medical University Institutional Review Board, which is guided by local policy, national law and the World Medical Association Declaration of Helsinki. For each type of blood component, the data included the total number of first transfusion episodes on patients without any transfusion history and the total number of subsequent transfusion episodes for those with transfusion history, as well as the total number of transfusion-related ATRs per blood component with respect to both first and subsequent transfusions. We can identify first transfusions because physicians and nurses routinely solicit transfusion history from patients or family members and check hospital databases and medical records. We have defined a transfusion episode as any number of units of the same type administered within 24 h of each other. Therefore, if a patient with no transfusion history received more than one type of blood component within 24 h, a 'first transfusion' for each type of blood component was recorded. Any blood components thereafter would belong to a subsequent (non-first) transfusion.

Physicians and nurses monitored patients after the start of each transfusion for the occurrence of any ATRs and reported to the transfusion medicine service of each hospital whether or not an ATR had occurred. ATRs were investigated by a physician trained in transfusion medicine, and additional clinical and biological information was collected to facilitate diagnosis and assessment of severity. Adverse reactions were deemed ATRs according to professional assessment of imputability based on clinical and laboratory data.

A standard ATR assessment form included the following 16 items as shown in Table 1. A febrile non-haemolytic transfusion reaction (FNHTR) was diagnosed if fever, chills/rigours and feverishness (subjective feeling of warmth or glow) occurred during or within 4 h following transfusion without any other cause such as haemolysis, bacterial contamination or underlying disease. Allergic reactions consisted of skin symptoms or signs such as pruritus, skin rash and urticaria within 6 h of transfusion, after other potential etiologies of an allergic reaction were excluded. Any additional findings or comments, including suspected transfusion-related acute lung injury (TRALI), transfusion-associated circulatory overload (TACO) or transfusion-transmitted viral and bacterial infection, could be entered as free text, and were later analysed. The definitions of all signs, symptoms and specific clinical syndromes used by the Japan Society of Transfusion Medicine and Cell Therapy (JSTMCT) are based on

Table 1 Signs, symptoms and clinical diagnoses of ATRs

Signs/Symptoms	Clinical diagnoses
Fever (more than 38°C and more than 1°C increase after a transfusion)	Serious allergic reaction
	TRALI
Chills/Rigor	TACO
Feverishness (feeling of warmth or glow)	PTP
Pruritus	GVHD
Skin rash	Haemolytic transfusion reaction
Urticaria	Acute
Respiratory distress	Delayed
Nausea/Vomiting	Transfusion-transmitted infection
Headache	HBV
Chest/flank/back pain	HCV
Hypotension (a decrease of more than 30 mmHg after a transfusion)	HIV
	Bacteria
Hypertension (a increase of more than 30 mmHg after a transfusion)	Others
Tachycardia	
Vein pain	
Disturbance of consciousness	
Haemoglobinuria	
Others	

ATR, adverse transfusion reaction; TRALI, transfusion-related acute lung injury; TACO, transfusion-associated circulatory overload; PTP, post-transfusion purpura; GVHD, graft-versus-host disease; HBV, hepatitis B virus; HCV, hepatitis C virus; HIV, human immunodeficiency virus.

documents issued by the International Society of Blood Transfusion (ISBT) Working Party for Haemovigilance [10], which also defined the criteria for grading the severity of ATRs, as follows: grade 1, the absence of immediate or long-term consequences; grade 2, long-term morbidity; grade 3, immediate vital risk; and grade 4, death of the recipient. Serious ATRs were defined as grade 2 or higher according to documents issued by the ISBT Working Party for Haemovigilance.

Blood components

Blood collection, preparation and testing were performed according to protocols of the Blood Service Headquarters of the JRCS. Types of blood donation were 200 ml or 400 ml of whole blood and apheresis of platelets or plasma. Since January 2007, only prestorage leucoreduced blood components ($<1 \times 10^6$ leucocytes per unit) have been manufactured. After venipuncture, the first 25 ml of blood is diverted to decrease the risk of bacterial contamination, although not all units were tested for bacterial contamination. All blood components were screened using serological testing for infectious diseases. Furthermore, all blood components were screened using 20-minipool nucleic acid testing to reduce the risk of transfusion-transmitted infectious diseases (hepatitis B virus, hepatitis C virus and human immunodeficiency virus). RBCs are prepared with the additive solution MAP (mannitol adenine phosphate) and stored for up to 21 days at 5°C. All PCs are collected from single donors by apheresis, suspended in 200 ml of plasma and stored for up to 4 days at 22°C with agitation. FFP is prepared from whole blood plasma or by apheresis from single donors. Final volumes of FFP derived from 200 to 400 ml whole blood donations are approximately 120 and 240 ml, respectively, whereas the volume of FFP derived from single donor apheresis is around 450 ml. All blood components excluding FFP are irradiated with 15–50 Gy to prevent transfusion-associated graft-versus-host disease.

Statistical analysis

Data were analysed for first transfusion episodes and for subsequent transfusion episodes. To calculate the frequency of ATRs, the number of confirmed ATRs was correlated with the total number of first and subsequent transfusion episodes. All statistical analyses were performed by the chi-squared test, with Yates's correction for continuity and/or a Student's t-test. P values below 0·05 were considered statistically significant.

Results

Basic transfusion data set

During this study, there were 7594 RBC, 2469 FFP and 2127 PC first transfusion episodes (Table 2). As for the

Table 2 Number of transfusions

	First transfusion	Subsequent transfusion
No. of transfusion episodes		
RBC	7594	31 767
FFP	2469	6652
PC	2127	25 866
Gender ratio (female/male)		
RBC	0·8 (3380/4214)	0·6 (12 452/19 315)
FFP	0·7 (1031/1438)	0·6 (2463/4189)
PC	0·7 (896/1231)	0·6 (9533/16 333)
No. of units per episode[a]		
RBC	2·0	1·6
FFP	2·6	3·0
PC	1·2	1·1

[a]The values are averages.

gender distribution of first transfusion episodes, the female-to-male ratios for each blood component were at or near 0·7. On the subsequent transfusion episodes, the female-to-male ratios for each blood component were all 0·6. Thus, the gender distributions are quite similar for each blood component in both categories of transfusion episode. Furthermore, none of the differences in the number of units per episode for each blood component between first and subsequent transfusions was statistically significant.

ATRs following transfusion of blood components

As shown in Table 3, the incidence of ATRs to RBCs was significantly higher on first (1·08%) vs. subsequent (0·69%) transfusion episodes ($P = 0·0004$). Also, the incidence of ATRs to FFP was significantly higher on the first than on subsequent transfusion episodes: 2·84% vs. 1·91%, respectively ($P = 0·006$). Furthermore, although there was no significant difference in the incidence of ATRs to PC between first and subsequent transfusion episodes, the incidence of ATRs to PC trended higher on first vs. subsequent transfusion episodes (3·34% vs. 2·75%, respectively, but without statistical significance ($P = 0·10$).

When the incidence of ATRs to each blood component was investigated among males, the frequency of ATRs to RBCs on first transfusion episodes was found to be significantly higher than that on subsequent transfusion episodes ($P = 0·002$) (Table 3). The incidences of ATRs to FFP and PCs on first transfusion episodes were slightly higher than those on subsequent transfusion episodes. On the other hand, for females, the incidence of ATRs to FFP on first transfusion episodes was significantly higher than that on subsequent transfusion episodes ($P = 0·018$). Also, the incidences of ATRs to RBCs and PCs on first transfusion episodes were slightly higher than those on subsequent transfusion episodes among females.

Table 3 Incidence of ATRs on first transfusion and subsequent transfusion episode bases

	First transfusion		Subsequent transfusion		
	Number	Incidence (%)	Number	Incidence (%)	*P* value[a]
RBC					
Male	39	0·93	103	0·53	0·002
Female	43	1·27	117	0·94	0·08
Total	82	1·08	220	0·69	0·0004
FFP					
Male	34	2·36	75	1·79	0·18
Female	36	3·49	52	2·11	0·018
Total	70	3·24	127	1·91	0·006
PC					
Male	40	3·25	440	2·69	0·28
Female	31	3·46	271	2·84	0·30
Total	71	3·34	711	2·75	0·10

ATR, Adverse transfusion reaction.

[a]*P* values are for difference of incidences of ATRs between first transfusion and subsequent transfusion episodes.

Characteristics of clinical signs and symptoms associated with ATRs

When the incidence of various ATR types to RBCs was investigated, the frequency of FNHTR (0·43%) and other reactions (0·26%) on first transfusion episodes was found to be significantly higher than on subsequent transfusion episodes (FNHTR: 0·23%; $P = 0·001$, other reactions: 0·15%; $P = 0·03$) (Table 4). The first transfusion incidence of allergic reactions (0·47%) was slightly higher than for subsequent transfusion episodes (0·37%). Furthermore, first transfusion incidence of allergic reactions (2·51%) to FFP was significantly higher than that on subsequent transfusion episodes (1·65%, $P = 0·01$). On the other hand, there were no significant differences in the frequency of FNHTR, allergic reactions and others to PCs on first transfusion episodes vs. subsequent transfusion episodes (FNHTR: 0·33% vs. 0·23%, allergic reactions: 2·82% vs. 2·49%, others: 0·33% vs. 0·12%).

During the study, serious ATRs to RBCs and FFP were 3 (0·04%) and 4 (0·16%), respectively on first transfusion episodes (Table 4). On subsequent transfusion episodes, serious ATRs to RBCs, FFP and PCs were 5 (0·02%), 2 (0·03%) and 7 (0·03%), respectively. The proportions of serious ATRs among all ATRs to RBCs, FFP and PCs were low on first and subsequent transfusion episodes; thus, the majority of ATRs were not serious.

Discussion

One factor thought to influence the risk of ATRs is transfusion history. Despite this, previous reports about ATRs have not distinguished first from subsequent transfusions in their analysis. In the present study, incidences of ATRs to RBCs, FFP and PCs per first transfusion episode were 1·08%, 2·84% and 3·34%, respectively. On the other hand, the incidences of ATRs on subsequent transfusion episodes to RBCs, FFP and PCs were 0·69%, 1·91% and 2·75%, respectively. Thus, the risk of ATRs for patients with no transfusion history trended higher compared to patients who had already been transfused. In particular, the first transfusion incidences of ATRs to RBCs and FFP are significantly higher than those for subsequent

Table 4 Clinical characteristics of ATRs on first transfusion and subsequent transfusion episodes

	RBC			FFP			PC		
	First[a] *n* (%)	Subsequent[b] *n* (%)	*P* value[c]	First[a] *n* (%)	Subsequent[b] *n* (%)	*P* value[c]	First[a] *n* (%)	Subsequent[b] *n* (%)	*P* value[c]
FNHTR	33 (0·43)	73 (0·23)	0·001	7 (0·28)	6 (0·09)	0·07	7 (0·33)	60 (0·23)	0·35
Allergic reaction	36 (0·47)	108 (0·34)	0·09	62 (2·51)	110 (1·65)	0·01	60 (2·82)	643 (2·49)	0·31
Others	20 (0·26)	48 (0·15)	0·03	5 (0·20)	17 (0·26)	0·63	7 (0·33)	31 (0·12)	0·16
Serious ATR[d]									
Serious allergic reaction	2 (0·03)	5 (0·02)	0·28	4 (0·16)	2 (0·03)	0·18	0	7 (0·03)	0·46
TRALI	0	0	–	0	1 (0·01)	–	0	0	–
Delayed haemolytic reaction	1 (0·01)	0	–	0	0	–	0	0	–

ATR, Adverse transfusion reaction; FNHTR, Febrile non-haemolytic transfusion reaction.
The values are the number of ATRs (%).
[a]First transfusion episodes.
[b]Subsequent transfusion episodes.
[c]*P* values are for difference of incidences of ATRs between first transfusion and subsequent transfusion episodes.
[d]Serious ATRs were defined as grade 2 or higher according to documents issued by the ISBT Working Party for Haemovigilance and include serious allergic reaction, transfusion-related acute lung injury (TRALI) and haemolytic transfusion reaction.

transfusion episodes ($P < 0·01$). Furthermore, the first transfusion incidences of FNHTR to RBCs (0·43%, $P = 0·001$) and allergic reactions to FFP (2·51%, $P = 0·01$) were higher than those on subsequent transfusion episodes (RBCs: 0·23%, FFP: 1·65%). Therefore, the higher frequencies of ATRs to RBCs and FFP on the first transfusion vs. subsequent transfusions could be traced to the higher incidences of FNHTRs to RBCs and allergic reactions to FFP on the first transfusion.

Previous work has reported that the risk of ATRs for patients who had already been transfused trended higher compared to patients with no transfusion history [11]. In general, Japanese physicians and nurses must obtain transfusion histories from patients or their family members and the physicians in transfusion medicine check this history against hospital database records. Furthermore, they infer whether or not patients have received any previous transfusions based on the patients' diseases. Thus, in the present study, almost all patients in the first transfusion category are considered to be reliably categorized. Another possible reason for this discrepancy comes from a previous study that surveyed ATRs to only PCs and did not reach statistical significance in the incidences of ATRs for patients with no transfusion history vs. any transfusion history. Indeed, in the present study, although the first transfusion incidence of ATRs to PCs trended higher than for subsequent transfusions, the difference did not reach statistical significance ($P = 0·10$). It is suspected that although the data in this study were not analysed for the characteristics of transfused patients, such as underlying condition, age and pregnancy history, there are risks of ATRs on the first transfusion.

Heddle *et al.* [12, 13] have reported that FNHTRs to blood components are caused by white blood cell (WBC) antigen–antibody interaction and cytokines, such as interleukin (IL)-1β, IL-6 and tumour necrosis factor-α, released from WBCs and accumulated in blood components during storage. There is general agreement that prestorage leucoreduction will decrease the frequency of FNHTRs [14, 15]. However, leucoreduction dose not completely reduce the amount of CD154 (CD40 ligand), implicated in FNHTRs and elaborated by platelets. Thus, it is unlikely that leucocyte filtration by itself can completely eliminate the risk of FNHTRs. Furthermore, a recent study revealed that some cytokines start accumulating in stored whole blood within hours after donation [16]. In this study, although all blood components were leucocyte reduced before storage, a low rate of FNHTRs to blood components persisted both on first transfusion and subsequent transfusion episodes. In particular, the incidence of FNHTRs per first transfusion episode to RBCs, which contain relatively more WBCs than other blood components, was significantly higher than on subsequent transfusion episodes.

Furthermore, the number of RBC units per episode for the first transfusion was slightly more compared with that on subsequent transfusion episodes. In particular, the number of RBC units per episode for the first transfusion tended to be more than that on subsequent transfusion episodes for males (data not shown). Therefore, it is suspected that FNHTRs to blood components are not influenced by previous allogeneic exposure from transfusion and one risk of ATRs on the transfusion was correlated with the number of transfusion units per episode.

It is thought that allergic reactions to blood components are influenced by previous allogeneic exposure from transfusion. Tobian *et al.* [17] described that patients must be exposed to plasma multiple times before having an allergic reaction. In addition, the incidence of ATRs per patient was influenced by the number of transfusions per patient [4, 11]. However, in this study, the incidences of allergic reactions to RBCs, FFP and PCs were 0·47%, 2·51% and 2·82%, respectively on the first transfusion though these patients were not previously exposed to allogeneic transfusion (Table 4). Furthermore, there were 6 serious allergic reactions associated with first transfusions. Putative mechanisms underlying allergic reactions include biological response modifiers (BRMs) such as inflammatory cytokines and chemokines that accumulate during storage and enter the circulation during transfusion, leading to allergic reactions [18–22]. On the other hand, Savage *et al.* [23] showed that certain donors donated PCs that resulted in an allergic reaction rate of 5·8%, which was greater than the overall incidence of allergic reactions (1·72%). A recent study observed 1616 (1·72%) allergic reactions among 93 737 transfusions and found that 30% of recipients with an allergic reaction had allergic reaction rates of more than 5% [23]. Furthermore, in 630 instances where split PCs were given to two patients in whom one had a reaction, there were only six instances where the other patient also had a reaction [23]. On the other hand, Ahmed *et al.* [24] reported that pregnancy history directly correlates with the risk of allergic reaction on initial transfusion. Indeed, although the data analysis in this study did not consider pregnancy for female patients, the incidence of ATRs (3·49%) per first transfusion episode to FFP was significantly higher than on subsequent transfusion episodes (2·11%, $P = 0·018$, Table 3). Thus, it is suspected that blood component factors, donor factors and patient factors including pregnancy may contribute to allergic reactions. Therefore, as the present study shows, risks of allergic reactions to blood component exist in patients without previous allogeneic exposure from transfusion.

The incidences of allergic reactions to blood components on the first transfusion were higher or almost the same compared to subsequent transfusion episodes. In particular, the frequency of allergic reactions to FFP (2·51%) on the first transfusion was significantly higher compared to subsequent transfusion (1·65%, $P = 0·01$). It is possible that this pattern is due to an increased use of premedication among patients with reactions to a previous transfusion. However, most studies performed to date have failed to demonstrate that premedication is effective in preventing allergic transfusion reactions [25–28]. Furthermore, in the present study, although most patients receiving RBCs and PCs were premedicated, the first transfusion incidences of allergic reactions to RBCs and PCs were not significantly higher than for subsequent transfusions. Another possibility is that serial exposure to blood components may desensitize recipients. A recent study revealed that recurrent exposure to PCs caused recipients to become less likely to experience allergic reactions [29]. Furthermore, experiments show a desensitization effect on repeat exposure to reconstituted donor serum [30]. It is thought that desensitization is mediated by two mechanisms, the suppression of proallergic innate effectors and the upregulation of regulatory T-cell activity. Proallergic innate effectors could undergo rapid desensitization against allergens [31]. In addition, functional allergen-specific regulatory T cells can attenuate allergic responses through suppression of mast cells, basophils and eosinophils; suppression of allergen-specific T cells; and reduction of IgE production [31]. Indeed, Jo *et al.* [32] showed the acquisition of tolerance to cow's milk allergens during childhood is associated with the suppression of proallergic innate effectors' activity and activity of regulatory T cells. Taken together, these findings help explain why the incidence of allergic reactions to blood components on first transfusions could exceed that of subsequent transfusions.

We conclude that there are risks of ATRs on the first transfusion as well as transfusions of patients with transfusion history. It is suspected that FNHTRs are not so much provoked by previous allogeneic transfusion exposure as by the number of transfusion units per episode. On the other hand, with regard to allergic reactions, although both atopic susceptibility in the recipient as well as particular donor and component characteristics are risk factors, the first transfusion itself appears to be an important risk. Thus, despite the limitations of this study, it suggests the need for more elaborate analyses of patient data to further improve transfusion therapy. In particular, ATR risk factors elucidated here and elsewhere might refine clinical indications for washing PCs.

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Conflict of interest

The authors declare no conflict of interests.

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Novel swine model of transfusion-related acute lung injury

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BACKGROUND: Transfusion-related acute lung injury (TRALI) is a life-threatening complication of blood transfusion. Antibodies against human leukocyte antigens in donors' plasma are the major causes of TRALI. Several animal models of TRALI have been developed, and the mechanism underlying TRALI development has been extensively investigated using rodent models. Although sheep models of nonimmune TRALI have been developed, large-animal models of antibody-mediated TRALI are not yet available.

STUDY DESIGN AND METHODS: To develop a swine model of TRALI, male Clawn strain miniature pigs were used. A monoclonal antibody (MoAb) against swine leukocyte antigens (SLAs) Class I (4G8, 0.3 or 1.0 mg/kg body weight [BW]) and a control antibody (1.0 mg/kg BW) were injected into the peripheral vein after priming with or without 1 μg/kg BW lipopolysaccharide (LPS; n = 3 each). Lung injury was assessed using PaO_2/FiO_2 (P/F) ratio and by chest X-ray imaging. Histopathologic analysis was also conducted.

RESULTS: Lung injury could be induced by injecting 4G8 at an amount of 1.0 mg/kg BW, after LPS. The P/F ratio 90 minutes after the administration of 4G8 significantly decreased ($p < 0.05$). Bilateral infiltration was shown in chest X-ray imaging. Lung injury was confirmed by histopathologic analysis.

CONCLUSION: Lung injury in pigs was successfully induced by anti-SLA MoAb. Priming with LPS is a prerequisite for inducing lung injury and the amount of the antibody is a critical condition.

Transfusion-related acute lung injury (TRALI) is a life-threatening complication of blood transfusion. Currently, the mechanism of TRALI induction is considered to be a two-hit event model. The first hit is the neutrophil priming elicited by the patient's underlying condition leading neutrophils

ABBREVIATIONS: ARDS = acute respiratory distress syndrome; BAL = bronchoalveolar lavage; BW = body weight; LPS = lipopolysaccharide; P/F ratio = PaO_2/FiO_2 ratio; SLA(s) = swine leukocyte antigen(s).

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sequestrated into the lung tissue. In the second hit, two different mediators have been proposed, namely antibodies and biologic response modifiers such as lysophosphatidylcholine. In the former, antibodies against human leukocyte antigens (HLAs) and/or human neutrophil antigens (HNAs) in donors' plasma react with the cognate antigens on the patient's white blood cells (WBCs) or other cell types activating them to cause the impairment of the endothelial barrier function, which results in the alveolar edema. In the latter, biologic response modifiers in stored blood products facilitate the activation of neutrophils, leading to endothelial leakage and alveolar edema.[1,2] Several animal models of TRALI using mice, rats, and sheep have been developed, and the underlying mechanism of TRALI induction has been extensively investigated particularly using rodent models.[3-9] Some treatment options, such as aspirin, have been shown to ameliorate lung injury in mice, although the mechanisms of action of anticoagulants are not fully clarified. However, in humans, the use of aspirin in TRALI or acute respiratory distress syndrome (ARDS) has shown contradictory findings.[10,11] Other promising treatment options, such as intravenous (IV) immunoglobulin or DNase, have also been proposed for the prevention and treatment of TRALI in mice.[12-14] Because the incidence of TRALI is low and prospective human studies are difficult to conduct, large-animal models mimicking human TRALI might be useful for searching for treatment options. Because no clinically proven treatment has been established for the treatment of ARDS, and so far, no specific treatment has been proposed for human TRALI, establishing a large-animal model of TRALI might facilitate the search for the novel treatments of TRALI. Furthermore, a large-animal model of nonimmune TRALI using sheep has been developed by an Australian group; however, antibody-mediated TRALI in a large-animal model is not yet available. Here, we report the establishment of a novel swine model of TRALI by the sequential administration of a low dose of lipopolysaccharide (LPS) and a monoclonal antibody (MoAb) against swine leukocyte antigen (SLA) Class I.

MATERIALS AND METHODS

Reagents

The 4G8 antibody against SLA Class I antigens was produced from the cultured hybridoma cell line as described previously.[15] LPS (*Escherichia coli* 055:B5) was purchased from Sigma-Aldrich (Tokyo, Japan).

Animals

After obtaining approval from the University of Tokyo Ethics Committee for Animal Experiments, conventional miniature pigs (Clawn strain, male, 9-10 months, 19.1-25.1 kg) were purchased from Japan Farm Clawn Institute (Kagoshima, Japan) and used for the experiment.

Anesthesia and catheterization

After induction of anesthesia by an intramuscular injection of 2.0 mg/kg body weight (BW) xylazine hydrochloride (Bayer, Leverkusen, Germany), 0.5 mg of midazolam (Astellas, Tokyo, Japan), and 0.5 mg of atropine sulfate, the pigs were intubated and mechanically ventilated with inhalation anesthesia with isoflurane (Dainippon Sumitomo Pharma, Osaka, Japan) using an anesthesia apparatus (ACOMA pro-45V, Acoma Medical Industry Co., Ltd., Tokyo, Japan). Mechanical ventilation was performed with the following settings: tidal volume, 10 mL/kg at 12 breaths/min; inspiration-to-expiration ratio, 1:1.9; FiO_2 levels, 30% to 35%.

A right or left carotid artery line was inserted, a Swan-Ganz catheter was introduced into the right or left jugular vein (Swan-Ganz standard thermodilution pulmonary artery catheter 174HF7, Edwards Lifesciences, Tokyo, Japan), and lactated Ringer's solution was dripped IV through the ear vein at a speed of 100 mL/hr.

Endotoxin and MoAb infusion

After a stabilization period of 10 to 20 minutes, 1 μg/kg BW LPS in 30 mL of normal saline or control normal saline was infused via the peripheral line over 30 minutes. After further stabilization for another 30 minutes, the pigs were administered either 0.3 (low dose) or 1.0 (high dose) mg/kg BW 4G8 SLA Class I MoAb or K-14 as the control MoAb (antibody to human Kell antigen: the same subclass as 4G8: IgG1) via the peripheral line over 120 minutes. Arm 1, LPS + 4G8 0.3 mg/kg BW; Arm 2, LPS + 4G8 1.0 mg/kg BW; Arm 3, NS + 4G8 1.0 mg/kg BW; Arm 4, LPS + K-14 1.0 mg/kg BW; and Arm 5, NS + K-14 1.0 mg/kg BW (n = 3 for each arm).

Neutrophil depletion

Cyclophosphamide was administrated IV through the ear vein 4 days before the experiment. The neutrophil count of cyclophosphamide-treated pigs before the experiment was one-third to one-fourth of that of nontreated pigs. Thereafter, the procedures similar to animals in Arm 2, except for the additional 60 minutes follow-up time after each experiment, were performed.

Physiologic monitoring

Chest X-ray images were obtained at the beginning and at the end of the experiment (VPX-40, Toshiba, Tokyo, Japan; Regius Model 110A, Konica Minolta, Tokyo, Japan). C-arm X-ray images were monitored every 30 minutes (ARCADIS

Avantic, Siemens, Tokyo, Japan). Systemic arterial pressure, pulmonary artery pressure, electrocardiography findings, and percutaneous oxygen saturation level were continuously monitored and recorded (BSM-4103, Nihon Kohden, Tokyo, Japan). Cardiac output was also measured by the thermodilution method using hemodynamic monitor (Vigilance II, Edwards Lifesciences, Irvine, CA).

Bronchoalveolar lavage

A bronchoscope was inserted via an intubation tube using a bifurcated device, and bronchoalveolar lavage (BAL) was performed in one of the upper right lobes three times using 10 mL of normal saline before the experiment and another BAL was performed in another right upper lobe after the experiment. Cytospin from BAL fluid was prepared; the number of neutrophils was counted under a microscope and the percentage was calculated.

Blood sampling

Arterial blood samples were taken at baseline and every 30 minutes thereafter until the end of the experiment and analyzed using a blood gas analyzer (cobas b 121, Roche, Tokyo, Japan). Peripheral whole blood count with the percentages of neutrophils and platelet (PLT) count were analyzed using a differential hematology analyzer (VETSCAN HM II, Abaxis, Union City, CA) every 30 minutes.

Histopathologic examinations

After an experiment was finished, the pigs were humanely euthanized with a KCl infusion. The lungs were excised and fixed with formaldehyde solution and stained with hematoxylin-eosin. The lung samples were also immunostained with Simple Stain Rat MAX PO (MULTI), (the labeled polymer prepared by combining amino acid polymers with peroxidase [PO] and secondary antibody which is reduced to Fab' fragment) (Nichirei Biosciences, Tokyo, Japan) to determine the deposition sites of the 4G8 MoAb. Furthermore, immunofluorescence staining with donkey anti-mouse IgG (Alexa594) was performed for the detection of the precise localization of 4G8 antibody using serial section of the specimens.

Statistical analysis

For the comparison of P/F ratio between before (Time 0 min) and after (Time 180 min) the experiment in each group, paired t test was utilized. Significance was set at a p value of less than 0.05.

RESULTS

Development of lung injury in pigs

First, we aimed to induce the development of lung injury only by the administration of a MoAb to the SLA Class I, namely, 4G8. The reactivity of 4G8 antibody and K-14 control antibody to pig WBCs is shown in Fig. 1A. The 4G8 MoAb at 1 mg/kg BW administered through the peripheral vein over 2 hours caused no deterioration of respiratory function, although the peripheral neutrophil count in peripheral blood decreased and the pulmonary artery pressure was slightly raised (Fig. 1B, Arm 3; Fig. 3, Arm 3; Fig. 4, Arm 3).

We then investigated the administration of LPS as a priming agent at a dose not sufficient to cause lung injury by itself, followed by the administration of the anti-SLA. In approximately 90 minutes after the start of infusion of the 4G8 antibody, the P/F ratio started to decrease to values below 300, which fulfilled the TRALI criteria (Fig. 1B, Arm 2). At almost the same time, we detected bilateral infiltration on the chest X-ray images. The bilateral infiltration found on the chest X-ray images could be clearly observed after the 4G8 antibody had been completely infused (Fig. 2). This deterioration of respiratory function was not seen in Arm 1, where the dose of the 4G8 antibody was 0.3 mg/kg BW (Fig. 1B, Arm 1).

Although data of dead pigs were not included in the result, two pigs died during this experiment. One was in Arm 1 (LPS + low-dose 4G8), and the other was in Arm 3 (NS + high-dose 4G8). These pigs died within 60 minutes after starting 4G8 antibody infusion without any deterioration of respiratory function. The cause of death of these pigs might be cardiac impairment because of rapid increase of pulmonary arterial pressure.

Decreased WBC counts and PLT counts in peripheral blood

We found that the administration of 1 μg/kg BW LPS did not cause deterioration of the respiratory function, as determined from P/F ratio and chest X-ray images. After IV administration of LPS, the WBC count in peripheral blood decreased and the PLT count in peripheral blood also decreased; these counts further decreased after the administration of the 4G8 antibody (1 mg/kg), although these decreases were not significant (Fig. 3).

Physiologic findings

The systolic blood pressure, mean pulmonary artery pressure, heart rate, and cardiac output during the course of the experiment are shown in Fig. 4. Although the total fluid volumes infused in the pigs in each experiment were similar, there were individual variations of the cardiac function of the pigs. Thus, we continuously monitored heart rate, electrocardiography findings, blood pressure, pulmonary artery pressure, and also cardiac output every 30 minutes. Administration of LPS or the 4G8 antibody resulted in the increase in mean pulmonary artery pressure and the decrease in cardiac output, but both the

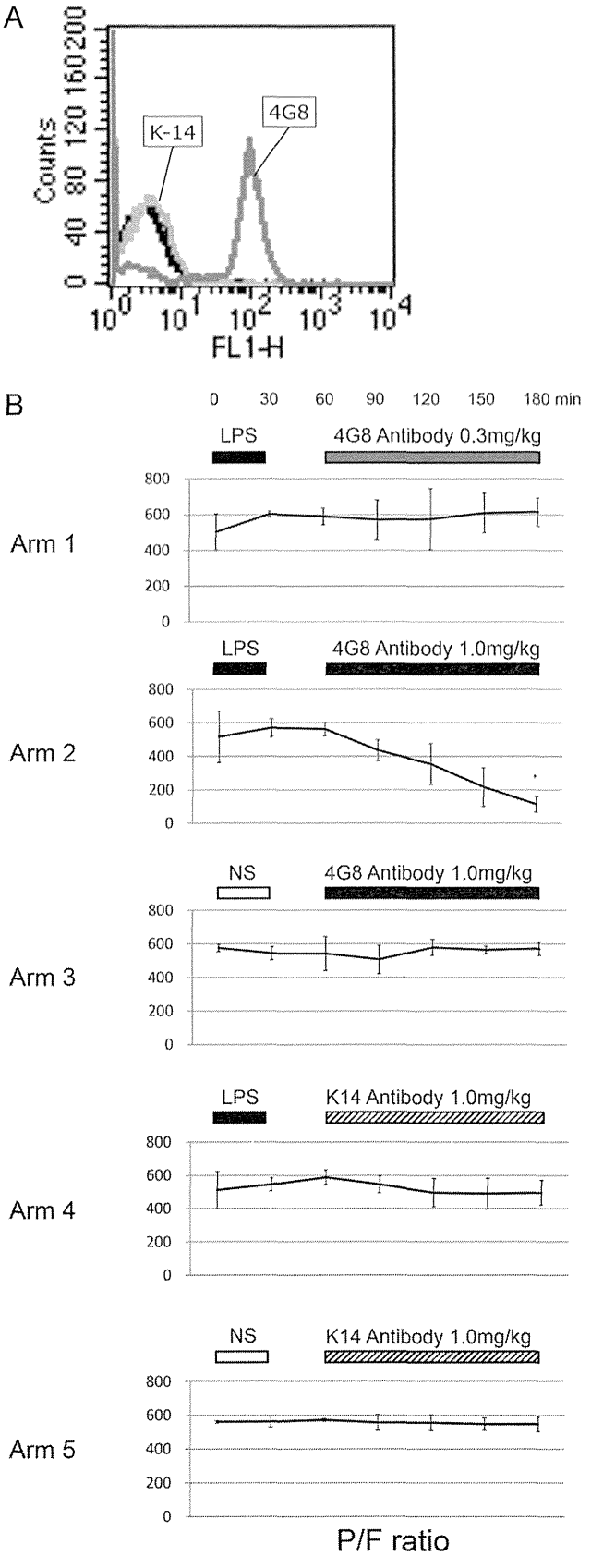

Fig. 1. (A) The reactivity of 4G8 antibody and K-14 control antibody to porcine WBCs is analyzed by a flow cytometer. (B) P/F ratio every 30 minutes is plotted. In Arm 2, P/F ratio significantly decreased 120 minutes after administration of the 4G8 antibody ($p < 0.05$, compared with other arms). Data represent the means ± SD.

systolic blood pressure and the heart rate were nearly stable during the experiments.

Neutrophil count in BAL cytospin preparation

Comparison of the percentage of neutrophils in the BAL fluid among the arms is shown in Table 1. The percentage of neutrophils was highest in Arm 2.

Histopathologic examination

Compared with the normal pig lungs (Fig. 5A) or the lungs from animals in Arm 5 (NS + K14 control antibody; Fig. 5F), in Arm 4 (LPS + K14 control antibody) a slightly increased neutrophil count was observed (Fig. 5E). The neutrophil count was high in Arm 1 (LPS + 4G8 SLA antibody 0.3 mg/kg BW), but the apparent alveolar effusion or bleeding was not detected (Fig. 5B). Evident neutrophil accumulation, alveolar effusion, and alveolar bleeding were detected in Arm 2 (LPS + 4G8 SLA antibody 1.0 mg/kg BW; Fig. 5C), and the deposition of the MoAb was detected along the alveolar wall (Fig. 5G). No apparent neutrophil accumulation was detected in Arm 3 (NS + SLA antibody 1.0 mg/kg BW; Fig 5D), but the deposition of the MoAb was detected by immunostaining (Fig. 5H). For further elucidation of the precise localization of the infused 4G8 antibody, immunofluorescence staining using the serial sections of lung specimens was conducted, which revealed its localization on the surface of macrophages and neutrophils, as well as on vascular endothelial cells and alveolar epithelial cells.

Neutrophil depletion

Ten pigs were used for the neutrophil depletion experiment, by infusion of cyclophosphamide; however, pneumonia developed in two pigs before the experiment and five pigs died during the experiment probably dependent on the rapid escalation of pulmonary artery pressure immediately after infusion of 4G8 antibody, without any radiologic evidence of lung injury. Thus, the experiments could be completed in only three pigs. Neutrophil depletion before the experiment could have prevented the development of lung injury in one pig and delayed its development in two pigs (Figs. 6A-6C).

DISCUSSION

In this study, we established a large-animal model of antibody-mediated TRALI, using Clawn strain miniature

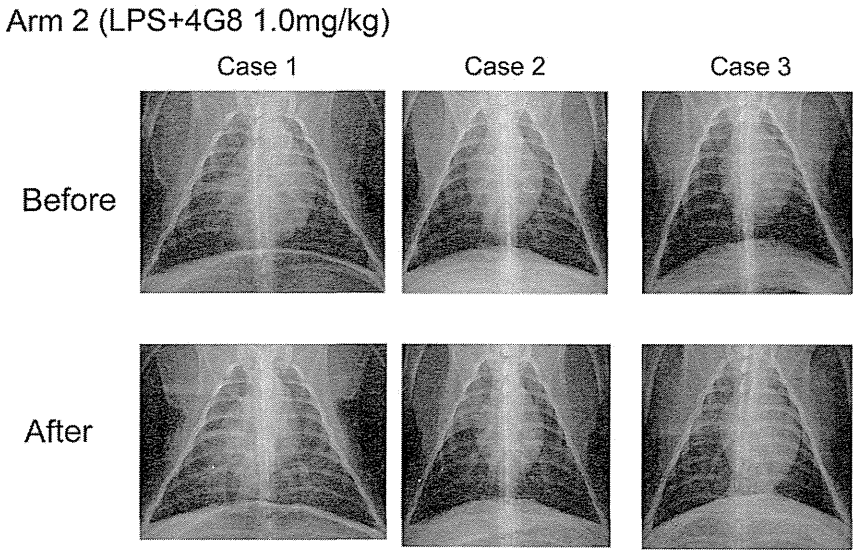

Fig. 2. Chest X-ray images of the before and after images of the three individual animals in Arm 2 (LPS + 4G8 antibody 1.0 mg/kg BW) are shown.

pigs. We observed that the anti-SLA alone was not sufficient for inducing clinically evident lung injury and that the priming with a small dose of LPS, which by itself did not induce lung injury, was a prerequisite. Moreover, the amount of the monoclonal SLA antibody was critical for the induction of clinical worsening of the respiratory function. To the best of our knowledge, this is the first reported large-animal model of antibody-mediated TRALI, which was confirmed by chest X-ray imaging. The presence of lung injury was also confirmed by the accumulation of neutrophils in the BAL fluid and by histopathologic analysis.

The incidence of TRALI is very low, making prospective randomized trials in humans difficult to conduct. It seems reasonable to search for treatment options for TRALI using appropriate animal models. To find an effective treatment for TRALI, a large-animal model is more advantageous in terms of the feasibility of continuous monitoring of hemodynamic changes and lung function than rodent models. In this study, we were able to investigate in detail the time course of physiologic changes such as the partial pressure of arterial oxygen, pulmonary arterial pressure, systemic blood pressure, and blood cell count. Furthermore, we were also able to monitor chest X-ray imaging findings during the experiment without requiring the repositioning of the pigs, because they could be anesthetized in the supine position. Pigs are advantageous because other large-animal models, such as sheep, need to be placed in the lateral decubitus position during the experimental procedure, which is not suitable for monitoring lung injury by chest X-ray imaging.

Pigs are generally much more sensitive to LPS than rodents. In the swine sepsis model of ARDS, the administration of doses as low as 0.1 μg/kg BW LPS resulted in pulmonary hypertension, which induces right heart failure before the deterioration of pulmonary gas exchange. A previous study has shown that the infusion of increasing doses of LPS successfully induces progressive ALI and prevents early pulmonary hypertension in pigs.[16] In our study, the administration of 1 μg/kg BW LPS over 30 minutes resulted in the early increase in pulmonary artery pressure and substantial decrease in cardiac output without affecting the respiratory function. We observed that the number of peripheral granulocytes markedly decreased after LPS administration, which might reflect granulocyte sequestration to capillary blood vessels. The deterioration of respiratory function that mimics TRALI could be induced only by the administration of a low-dose LPS followed by a high dose of monoclonal SLA antibody. Neither the sequential administration of LPS and the control MoAb, nor that of LPS and a low dose of anti-SLA MoAb, nor the administration of a high dose of anti-SLA MoAb alone could induce the similar clinical conditions. The prerequisite of the priming with LPS in this experiment is in agreement with the observation that sepsis is one of the major risk factors for the development of human TRALI.[17,18] These observations support the two-event model of ARDS advocated by Salzer and McCall[19] and also corroborate our previous observation that the strength of an anti-HLA is implicated in the development of TRALI.[20]

Two types of in vivo rodent model of antibody-mediated TRALI, the mouse and rat models, have been established and extensively studied so far. Both types of model are generated by an IV injection of monoclonal anti-major histocompatibility complex (MHC) Class I antibodies in the LPS-treated animals.[5,6] In both types of models, histopathologic changes mimicking those in human TRALI were observed in the lungs; however, the time course and the physiologic findings were not described in previous reports. The basic difference between these two types of model is the localization of the deposition of anti-MHC Class I MoAbs. In a mouse model, the anti-MHC Class I antibody 34-1-2 was found to diffusely bind to the microvasculature, whereas in a rat model, the anti-MHC Class I antibody OX27 was found to bind to neutrophils and not to endothelial cells. The interesting observations in the mouse model are that female mice do not develop lung injury and that complement activation rather than activation of the Fcγ receptor is critical for the induction of lung injury.[8] Moreover, there seems to be a great variability in the reactivity of different

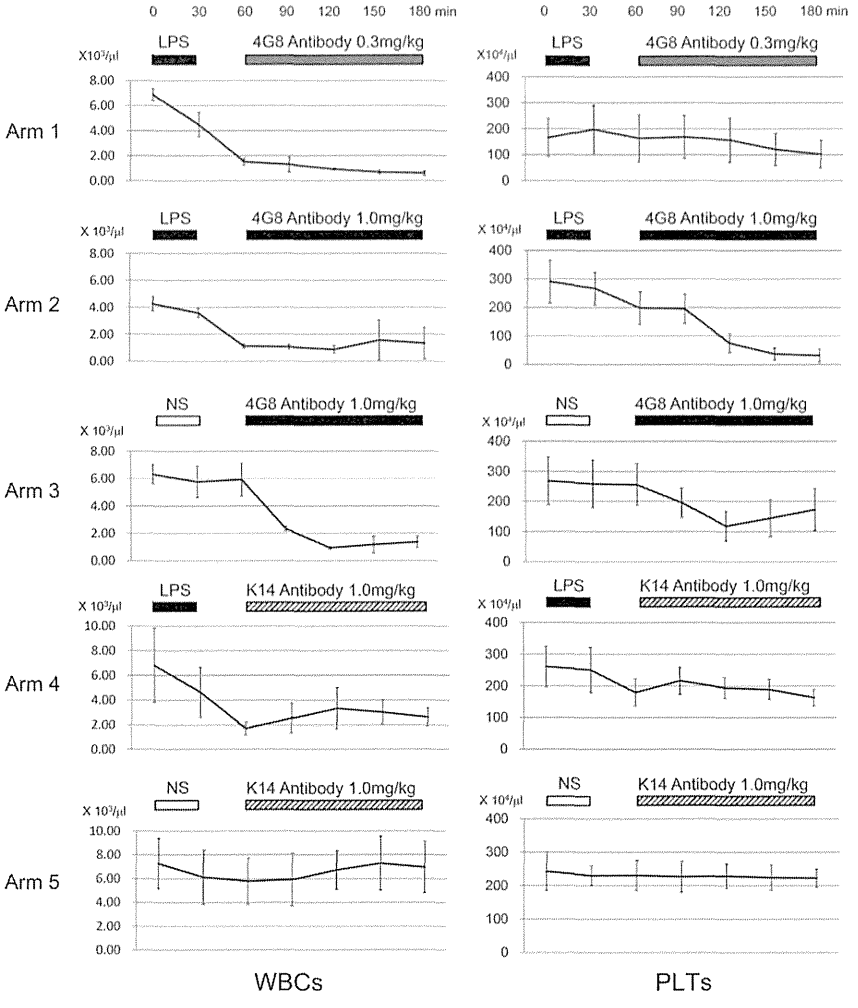

Fig. 3. WBC count and PLT count every 30 minutes are plotted. Data represent the means ± SD.

TABLE 1. % BAL neutrophils

Arm	Pre	Post
1, LPS + 4G8 0.3	0	1.5
	1.5	1.5
	2.0	9.0
2, LPS + 4G8 1.0	4.0	35.0
	5.5	21.0
	6.0	16.5
3, NS + 4G8 1.0	3.0	3.0
	2.0	3.5
	1.5	3.0
4, LPS + K14 1.0	0.5	3.5
	6.0	5.5
	0	4.0
5, NS + K14 1.0	0	3.5
	0	8.0
	0.5	9.0

MoAbs. In this swine model, other MoAbs have not been tested yet. We believe that it is very important to develop a reproducible model for investigating the efficacy of treatment, particularly a model that allows the accurate monitoring of physiologic variables. In this regard, although rodent models have many benefits for the study of the pathogenesis of TRALI, their applicability to the investigation of the clinical course of TRALI, particularly in terms of the implementation of preventive or therapeutic measures, has some limitations.

An ovine model of nonimmune TRALI, in which infusion of LPS is followed by that of a 5-day-old supernatant of human PLT concentrates, has been reported.[7] One of the advantages for the sheep model is the similarity of coagulation system to human, which will allow for investigation of the effects of the coagulation system on innate immunity involved in ALI.

The role of neutrophils in the pathogenesis of lung injury has not been fully elucidated. In this swine model, cyclophosphamide infusion 4 days before the experiment resulted in the mild decrease in the neutrophil count before the infusion of 4G8 antibody; however, out of 10 pigs two developed pneumonia, and five died during the experiment immediately after the infusion of 4G8. The deaths were attributed to the rapid decrease in systemic blood pressure accompanied by persistent high pulmonary arterial pressure, which might have been exacerbated by the administration of cyclophosphamide. Thus, substantial immunologic changes seem to have occurred by the administration of cyclophosphamide.

Immunofluorescence staining of the lung specimens revealed localization of the 4G8 antibody on the surface of intraalveolar macrophages and neutrophils as well as on alveolar epithelial cells and vascular endothelial cells. From these findings, we speculated that 4G8 bound to the SLA Class I expressed on intravascular macrophages, neutrophils, and vascular endothelial cells, leading to an increase of vascular permeability, enabling the penetration of the antibody to the alveolar space, where they react with alveolar epithelial cells. Because SLA Class I antigen is expressed on almost all cells, the deposition of 4G8 on various types of cells seemed to be reasonable. This observation is similar to the recent findings of HNA-3a–mediated TRALI, where HNA-3a recognizes antigens on endothelial cells as well as on neutrophils.[21] From the present swine model of TRALI, the precise role of neutrophils could not explicitly be determined; however,

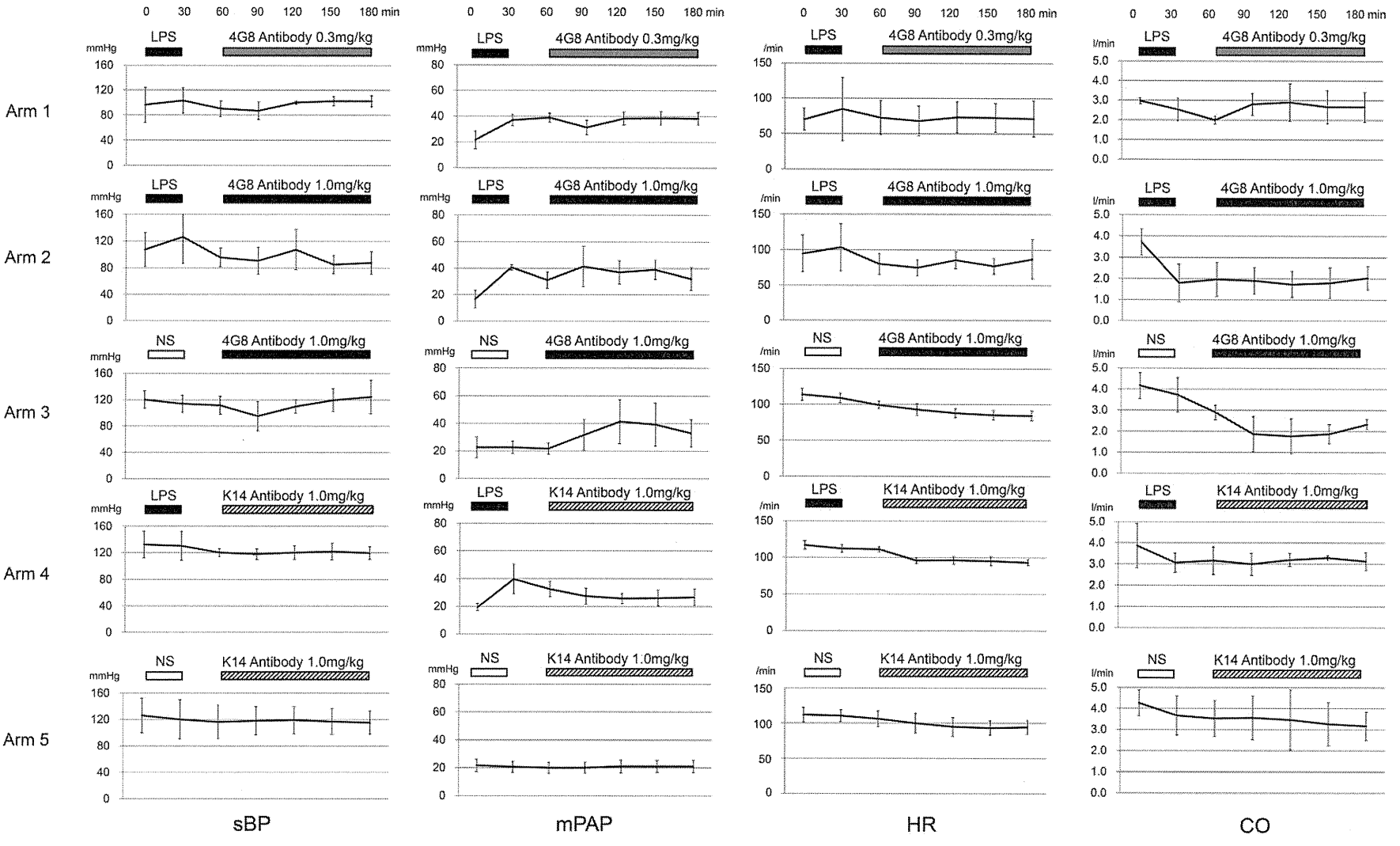

Fig. 4. Systolic blood pressure (sBP), mean pulmonary artery pressure (mPAP), heart rate (HR), and cardiac output (CO) every 30 minutes are plotted. Data represent the means ± SD.