



Curability and transferability of atopy with allogeneic hematopoietic cell transplantation

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Abstract

Atopy is excessive production of IgE in response to allergens. We evaluated in patients undergoing allogeneic hematopoietic cell transplantation (HCT) the following hypotheses: (1) Atopy is “curable” in atopic patients receiving HCT from a nonatopic donor (D-R+), and (2) Atopy is transferable from atopic donors to nonatopic recipients (D+R-). Atopic patients with atopic donors (D+R+) and non-atopic patients with non-atopic donors (D-R-) served as controls. We measured levels of multiallergen-specific IgE (A-IgE, atopy defined as ≥ 0.35 kU_A/L) in sera from 54 patients and their donors pre HCT and from the patients at ≥ 2 years post HCT. Only 7/12 (58%) D- R+ patients became nonatopic after HCT. Only 1/11 (9%) D+R- patients became atopic. Eleven of 13 (85%) D-R- patients remained nonatopic. Unexpectedly, 11/18 (61%) D+R+ patients became nonatopic. In conclusion, contrary to our hypothesis and previous reports, the “cure” of atopy may occur in only some D-R+ patients and the transfer of atopy may occur rarely. The “cure” may not be necessarily due to the exchange of atopic for nonatopic immune system, as the “cure” may also occur in D+R+ patients.

Introduction

Excess of allergen-specific IgE (A-IgE), here defined as serum level ≥ 0.35 kU_A/L (kiloUnits of antibody per liter of

serum), is referred to as atopy [1]. Most atopic patients have an allergic (atopic) disease like atopic asthma or rhinitis [2]. A-IgE is produced by plasma cells that have differentiated from allergen-specific B cells. The A-IgE production is regulated by both hematolymphatic cells like eosinophils or T cells [3–5] and by non-hematolymphatic cells like epithelial cells lining the respiratory tract [3, 6]. In allogeneic hematopoietic cell transplantation (HCT), recipient hematolymphatic cells (but not epithelial cells) are typically replaced with donor hematolymphatic cells. If hematolymphatic cells play the primary role in atopy, as generally thought to be the case [2], atopy should be transferable with HCT from an atopic donor to a nonatopic recipient and curable with HCT from a nonatopic donor to an atopic recipient. Consistent with this, there have been multiple case reports/small series published describing the transfer of atopic disease with HCT (if pre transplant the donor had an atopic disease and the recipient did not (D+R-) or, vice versa, the long-term remission (“cure”) of atopic disease (if pre transplant the recipient had an atopic disease and the donor did not (D-R+)) [7–17]. It is not known whether these represent exceptional cases versus whether atopic disease is always curable (in D-R+ patients) and always

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transferable (to D+R- patients). To answer these questions, large prospective studies will be needed. However, it may take many years before the results of the studies on the curability and transferability of atopic disease are known. In the meantime, it would be of interest to know whether atopy, a surrogate for atopic disease, is “curable” and transferable. That is the goal of this study.

Subjects and methods

We studied 54 allogeneic HCT recipients and their donors (Table 1). We collected serum pre transplant and at ≥ 2 years post transplant. We measured serum levels of multiallergen-specific IgE (hereafter “A-IgE”), and in some patients/donors also single allergen-specific IgE (hereafter “single A-IgE”). Atopy was defined as A-IgE or single A-IgE ≥ 0.35 kU_A/L. The rationale for evaluating atopy at ≥ 2 years post transplant was that pharmacologic immunosuppression was typically discontinued by 1 year, thus exposure to allergens in the second or subsequent years could lead to unopposed activation and differentiation of allergen-specific B cells into A-IgE plasma cells.

Results

“Curability” of atopy (D-R+ patients)

Of 12 D-R+ patients, seven (58%) were nonatopic at the last determination (2–10 years after HCT) whereas five (42%) were atopic (Fig. 1, top).

To evaluate whether the atopic status after HCT could be influenced by persisting recipient T or B cells, we determined T and B cell chimerism in 10 of the 12 D-R+ patients on whom cryopreserved MNCs from 1 year post transplant were available. No recipient T cells were detected in all ten patients. Likewise, no recipient B cells were detected. However, in one patient the B cell chimerism could not be determined due to insufficient quantity of B cells. In one of the two patients whose MNCs were not available, chimerism among total white blood cells had been determined by a clinical lab at 1, 4, 5, and 6 years post transplant. The percents of donor cells were 88%, 90%, 93%, and 96%, respectively. Interestingly, this patient had the highest A-IgE levels after HCT (dark blue line in Fig. 1, top). Collectively, despite a contribution of mixed chimerism in one patient cannot be excluded, most D-R+ patients with persisting atopy were complete chimeras.

Next, we assessed whether the persistence of atopy could be due to the donor being related, thus sharing genetic predisposition to develop atopy with the recipient. Of the seven D-R+ patients who became nonatopic, four (57%)

Table 1 Patient characteristics.

Number of patients	54
Recipient age at transplant, median (range)	46 (4–64)
Donor age at transplant, median (range)	34 (7–60)
Gender (Donor → recipient) (%)	
M → M	23 (42.6)
M → F	12 (22.2)
F → F	12 (22.2)
F → M	7 (13.0)
Diagnosis (%)	
ALL	7 (13.0)
AML	18 (33.3)
CLL/SLL	2 (3.7)
CML	6 (11.1)
Mantle cell lymphoma	2 (3.7)
MDS	7 (13.0)
Acute biphenotypic leukemia	1 (1.9)
Langerhans cell histiocytosis	1 (1.9)
CMML	2 (3.7)
MF	2 (3.7)
SAA	5 (9.3)
SCD	1 (1.9)
Donor/recipient atopic status (%)	
Donor+/recipient+	18 (33.3)
Donor−/recipient−	13 (24.0)
Donor+/recipient−	11 (20.4)
Donor−/recipient+	12 (22.2)
Donor type (%)	
HLA matched sibling	27 (50.0)
7–8/8 matched unrelated	25 (46.3)
Other ^a	2 (3.7)
Conditioning ^b (%)	
Fludarabine + busulfan	31 (57.4)
Fludarabine + busulfan + TBI (4 Gy)	12 (22.2)
Cyclophosphamide	5 (9.3)
Busulfan + cyclophosphamide	2 (3.7)
Other ^b	4 (7.4)
Graft type (%)	
PBSC	47 (87.0)
BM	7 (13.0)
GVHD prophylaxis (%)	
Thymoglobulin + methotrexate + cyclosporine	52 (96.3)
Other ^c	2 (3.7)
Developed significant GVHD ^d (%)	
Yes	26 (48.1)
No	28 (51.9)

M male, F female, ALL acute lymphoblastic leukemia, AML acute myeloid leukemia, CLL chronic lymphocytic leukemia, SLL small lymphocytic lymphoma, CML chronic myelogenous leukemia, MDS myelodysplastic syndromes, MPN myeloproliferative neoplasm, CMML chronic myelomonocytic leukemia, MF myelofibrosis, SAA severe aplastic anemia, SCD sickle cell disease, TBI total body irradiation, PBSC peripheral blood stem cells (filgrastim-mobilized), BM bone marrow, GVHD graft-versus-host disease.

^aOne 7–8/8 related nonsibling, one haploidentical related donor.

^bConditioning was myeloablative, except for five patients with SAA who received Cyclophosphamide 200 mg/kg and one patient with SCD who received TBI 3 Gy. Other refers to fludarabine + cyclophosphamide + melphalan ($n = 1$), fludarabine + thiotepa ($n = 1$), etoposide + TBI (12 Gy) ($n = 1$), TBI (3 Gy) ($n = 1$).

^cPosttransplant cyclophosphamide + mycophenolate mofetil + cyclosporine ($n = 1$), alemtuzumab + sirolimus ($n = 1$).

^dGrade 2–4 acute GVHD or moderate-severe chronic GVHD.

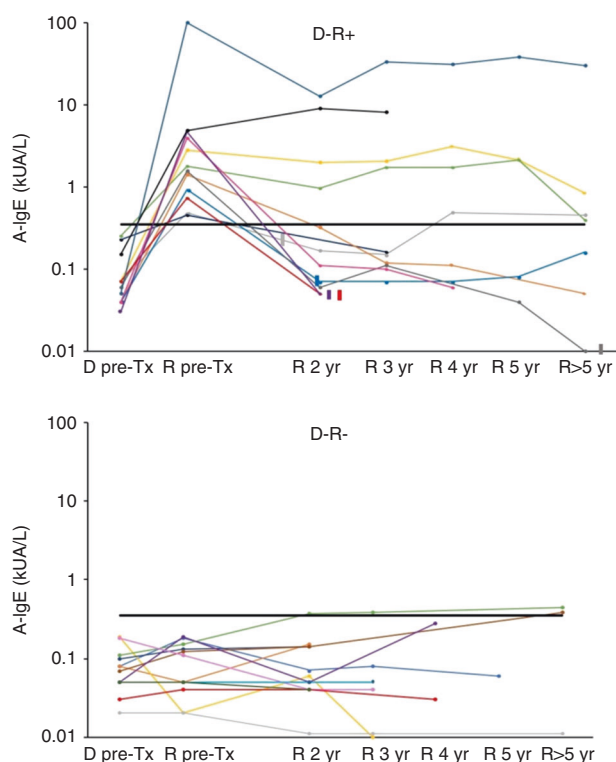


Fig. 1 A-IgE levels before and after HCT in D-R+ and D-R- patients. D-R+ patients are shown in the top panel and D-R- patients in the bottom panel. On the x-axis, D Pre-Tx denotes donor pre transplant, R Pre-Tx denotes recipient pre transplant, and R X yr denotes recipient at 2, 3, 4, 5, and >5 (6–14) years post transplant. Allergen-specific IgE (A-IgE) levels are shown on the y-axis and the horizontal line indicates the cutoff (0.35 kU_A/L) for atopy. For D-R+ patients who continued immunosuppressive therapy (IST) beyond 1 year post transplant, the end of IST is marked by a short vertical line. For D-R+ patients who discontinued IST in the first year, the end of IST is not marked. In two D-R- donors on whom pretransplant serum was not available, the A-IgE was measured in serum from 3 years post transplant (dark green and light blue lines).

had related donors and three (43%) had unrelated donors. Of the five patients with persistent or recurrent atopy, four (80%) had related donors and one (20%) had an unrelated donor (Fig. S1). Thus, donor relatedness was likely not the reason for persisting or recurrent atopy.

Next, we evaluated whether falsely negative A-IgE results in donor pretransplant serum could have accounted for the unexpected presence of atopy in the five D-R+ patients. The Phadiatop assay can give a falsely negative result if the concentration of IgE for only one of the 11 common allergens determined is in the atopic range [18–20]. This could lead to a D-R+ patient, in whom atopy is expected to persist, being falsely classified as a D-R- patient. Thus, in the pretransplant sera from the donors of the five D-R+ patient with persisting or recurrent atopy, we measured IgE specific for each one of the 11 allergens. In all 5 donors, the concentration of IgE for each allergen was <0.35 kU_A/L (Table S1). Thus, the unexpected

persistence of atopy was not due to a misclassification of the D-R+ patients.

It is also conceivable that a nonatopic donor was “pre-atopic”, i.e., was developing atopy which was not yet manifest as elevated A-IgE pre HCT but would become manifest by ≥2 years later, when we measured A-IgE in HCT recipients. To assess how likely it is for a nonatopic donor to become atopic, we measured A-IgE in 12 donors both pre HCT and median 8 years post HCT (range, 2–14 years). Nine of the 12 donors were nonatopic pre HCT. Eight of the nine (89%) nonatopic donors remained non-atopic and the remaining one donor had only mildly elevated A-IgE level at the post transplant determination (Fig. S2). Thus, it is unlikely that the unexpected persistence of atopy in some D-R+ patients was due to HCT from a “preatopic” donor.

Total IgE can be “spontaneously” elevated in the first several months post HCT, presumably due to post HCT immune dysregulation [21–23]. It is unknown whether this can also occur at ≥2 years post transplant and whether it applies to A-IgE. If yes, the elevated A-IgE in some of our D-R+ patients at ≥2 years could be due to the “spontaneous” IgE elevation rather than atopy persistence/recurrence. To evaluate this, we measured posttransplant A-IgE in the D-R- patients ($n = 13$). After HCT, 11 of them continued to have A-IgE in the nonatopic range, and two had only mildly elevated A-IgE (Fig. 1, bottom). Therefore, despite a minor contribution of “spontaneous” A-IgE elevation after HCT cannot be ruled out, it is unlikely that the persistence of atopy in all five D-R+ patients atopic after HCT represents the “spontaneous” IgE elevation.

An alternative explanation for the persistence of atopy in five versus the disappearance of atopy in seven D-R+ patients could be that atopy is not “curable”, however, it is masked in patients on long-term immunosuppressive therapy (IST), usually for GVHD [13]. This could possibly be the case, as none of the patients who were atopic post transplant were on IST for more than 1 year whereas five of the seven patients (71%) who became nonatopic were on IST more than 1 year (Fig. 1). However, this was not statistically significant (Fisher exact test). Likewise, GVHD itself is immunosuppressive [14]. Only one of the five patients (20%) with persistent atopy had significant GVHD (grade 2–4 acute or moderate–severe chronic GVHD at any time prior to the last A-IgE determination), whereas four of the seven patients (57%) who became nonatopic had significant GVHD (Fig. S3). This was also not statistically significant (Fisher exact test). Collectively, despite an influence of GVHD or IST cannot be ruled out, atopy did abate in one of four (25%) D-R+ patients without significant GVHD and off IST by 1 year.

In patients without significant GVHD or prolonged IST, immunity typically becomes normal by 2 years post

transplant, except CD4 T lymphopenia may persist longer [24]. Thus, it is conceivable that the patients who became nonatopic could not produce A-IgE due to a lack of CD4 T cells (which could help allergen-specific B cells to differentiate into A-IgE-producing plasma cells). However, the CD4 T cell counts were not significantly higher in the D-R+ patients with versus without atopy after HCT (Fig. S4). Thus, differences in the tempo of CD4 T cell reconstitution cannot explain why atopy abated in some versus persisted in other D-R+ patients.

Transferability of atopy (D+R- patients)

Only 1 of 11 (9%) D+R- patients became atopic after HCT (Fig. 2, top, and Fig. S5). The other ten (91%) patients remained nonatopic, at least within the follow up of 2–12 years post HCT.

As detailed in Supplementary Results, we could not find an explanation for why the transfer occurred in the one and not in the remaining ten patients.

Even in the D+R+ group, not every patient was atopic after the transplant. In 11 of 18 (61%) D+R+ patients, A-IgE levels declined into the nonatopic range after HCT (Fig. 2, bottom). The nonatopic state could not be attributed to long-term IST (Fig. 2, bottom), significant GVHD (Fig. S6) or low CD4 T cell counts (Fig. S7). Remotely, it could be attributed to spontaneous disappearance of atopy, as one of the three atopic donors on whom we measured A-IgE both pre HCT and at ≥ 2 years post HCT became nonatopic (Fig. S2). However, this donor had only mildly elevated A-IgE pre transplant.

Preliminary results on the curability and transferability of atopic asthma/rhinitis

Information on not only A-IgE but also symptoms of asthma and rhinitis was available on 11 prospectively studied patient–donor pairs (see Supplementary Methods). Of these, five were D-R+, two D+R-, one D+R+, and three D-R- (Table 2). Of the five D-R+ patients, one continued to have atopic asthma/rhinitis until at least 4 years post transplant and four became equivocal (three symptomatic with A-IgE <0.35 kU_A/L and one asymptomatic with A-IgE ≥ 0.35 kU_A/L). Of the two D+R- patients, one remained negative for atopic asthma/rhinitis until at least 3 years post transplant and one acquired atopic asthma by 7 years post transplant. The single D+R+ patient studied was negative for atopic asthma/rhinitis at 2 and 5 years post transplant, and in the third posttransplant year had mild rhinitis symptoms with nonatopic A-IgE (0.16 kU_A/L). All three D-R- patients remained negative for atopic asthma/rhinitis after HCT. Thus, given the small patient numbers and the large fraction of patients with equivocal posttransplant

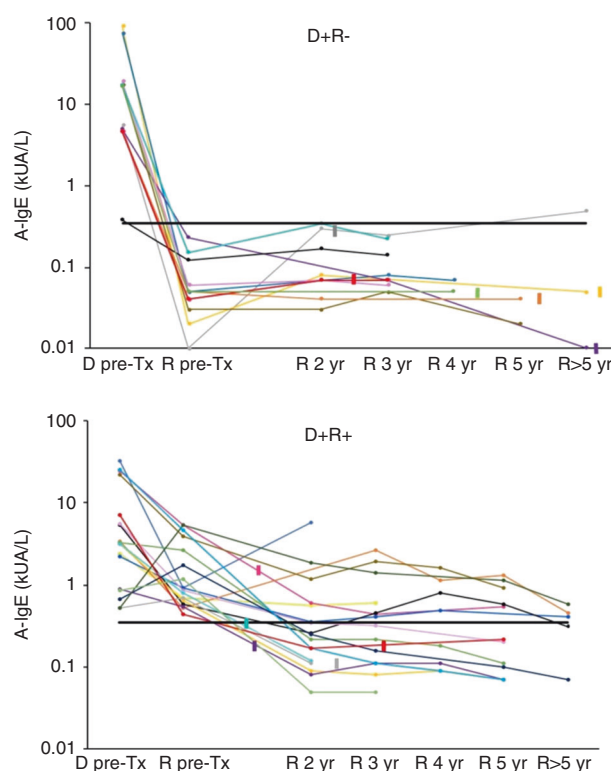


Fig. 2 A-IgE levels before and after HCT in D+R- and D+R+ patients. D+R- patients are shown in the top panel and D+R+ patients in the bottom panel. On the x-axis, D Pre-Tx denotes donor pre transplant, R Pre-Tx denotes recipient pre transplant, and R X yr denotes recipient at 2, 3, 4, 5, and >5 (6–12) years post transplant. Allergen-specific IgE (A-IgE) levels are shown on the y-axis and the horizontal line indicates the cutoff (0.35 kU_A/L) for atopy. For patients who continued immunosuppressive therapy (IST) beyond 1 year post transplant, the end of IST is marked by a short vertical line. For patients who discontinued IST in the first year, the end of IST is not marked. In one D+R- donor on whom pretransplant serum was not available, the A-IgE was measured in serum from 4 years post transplant (green line).

atopic asthma/rhinitis status, we can only conclude that atopic asthma/rhinitis is not always curable, and may be transferable to some but not all patients.

For additional results, see Supplementary Results online, Figs. S8, S9, S10, S11, and S12, Tables S2 and S3.

Discussion

Our results suggest that atopy is “curable” in only about half D-R+ patients. Surprisingly, the “cure” in the half patients cured may not be due to the replacement of atopic for nonatopic immune system, as atopy also abated in most D+R+ patients. As A-IgE also declined after autologous HCT (see Supplementary Results), conditioning itself could have a beneficial effect on atopy. The reason for this is not clear to us. However, it is reminiscent of the decline of

Table 2 Allergic disease status after HCT in 11 evaluable patients.

UPN	Category	Allergic asthma/rhinitis status after HCT				Asthma/rhinitis severity ^a		
		2 years	3 years	4–5 years	>5 years	Donor pre HCT	Patient pre HCT	Patient post HCT ^b
0895	D-R+	Pos	Pos	Pos	—	A0R0	A1R1	A1R1 (4 y)
1216	D-R+	Neg	Neg	Eq	—	A0R0	A0R1	A0R1 (5 y)
1467	D-R+	Eq	Eq	—	—	A0R0	A1R2	A2R2 (3 y)
1578	D-R+	Eq	—	—	—	A0R0	A0R1	A1R2 (2 y)
5001	D-R+	Eq	Pos	Eq	Eq	A0R0	A0R2	A0R0 (6 y)
0969	D+R-	Neg	Neg	—	—	A0R1	A0R0	A0R0 (3 y)
1193	D+R-	Pos	Pos	—	Pos	A0R1	A0R0	A2R0 (7 y)
1073	D+R+	Neg	Eq	Neg	—	A2R1	A0R1	A0R0 (5 y)
5002	D-R-	Neg	Neg	—	Neg	A0R0	A0R0	A0R0 (8 y)
5005	D-R-	—	Neg	—	—	A0R0	A0R0	A0R0 (3 y)
5006	D-R-	—	Neg	—	—	A0R0	A0R0	A0R0 (3 y)

Dash (—) denotes outcome not evaluated.

UPN unique patient number, *Pos* positive, *Neg* negative, *Eq* equivocal (symptoms of asthma or rhinitis in the preceding 12 months with A-IgE <0.35 kUA/L, or no symptoms of asthma or rhinitis in the preceding 12 month with A-IgE ≥kUA/L).

^aAsthma severity score (0–3) and Rhinitis severity score (0–3). For example, A0R3 means no asthma and severe rhinitis. For score definitions, see Supplementary Material Online.

^bAt the last time when the patient filled out the severity questionnaire. The time point is marked in parentheses (e.g., 5 y denotes 5 years after HCT).

autoantibodies in some studies of patients undergoing autologous HCT for autoimmune diseases [25, 26]. The phenomenon is referred to as “immune reset”, which may be related to thymic generation of a new T cell repertoire, posttransplant T cells secreting less proinflammatory cytokines than pretransplant T cells, or a fast regeneration of regulatory T cells [27]. The atopy “cure” in some D-R+ and some D+R+ patients could also be due to spontaneous disappearance of atopy which is known to occur with age even in the general population [28, 29]. However, that disappearance is more likely to occur over decades and not years of follow up [30, 31] as was the case in our study.

Our results also suggest that atopy is transferable rarely. This is contrary to multiple case reports on allergic disease transfer [7–12] and no report on the lack of thereof. This is also contrary to the only prospective study on atopic disease transfer reported so far, in which atopic rhinitis was transferred in five of five (100%) patients [13]. The reason for this is unclear. Perhaps, this may be related to ATG being used for GVHD prophylaxis in most of our patients but not in most patients in the published case reports or the prospective study. ATG is polyclonal, containing antibodies against multiple (>50) antigens expressed by virtually all subsets of hematolymphatic cells, including those involved in atopy like eosinophils, dendritic cells, T cells, B cells, or plasma cells [32, 33]. It is conceivable that ATG kills a substantial fraction of allergen-specific T or B cells transferred with the graft. However, among our eleven D+R- patients, one did not get

ATG and that patient was posttransplant nonatopic (green line in Fig. 2, top). Another potential reason for the transfer in previous reports whereas the relative lack of it in our patients could be our strict inclusion of only patients whose pretransplant blood was collected before induction chemotherapy/TKI or, if collected after chemotherapy/TKI, the inclusion of only patients with A-IgE >0.35 kUA/L. In the reported studies, the timing of the testing of patients for atopy was either after chemotherapy or not specified. Thus, D+R+ patients could have been misclassified as D+R- patients. This would invalidate the interpretation of atopic status post transplant as atopy transfer. For example, in our report of “transfer” of cat and dog dander atopy to a patient with acute myeloid leukemia in first remission, the pretransplant blood was obtained after induction and consolidation chemotherapy [34]. Likewise, in the prospective study of Agosti et al. [13], of the five patients who experienced “transfer” of atopic asthma/rhinitis, two underwent HCT for acute leukemia and three for chronic myeloid leukemia in chronic phase. Their atopic status was determined median 12 days before transplant, thus presumably after induction/consolidation chemotherapy or on/soon after discontinuation of busulfan (typical treatment of chronic myeloid leukemia in chronic phase before HCT in the 1970’s/1980’s, when the study was conducted).

Our study has limitations. Firstly, a reason for the persistence of atopy in some D-R+ patients could be the persistence of recipient A-IgE plasma cells, as plasma cells may be long-lived and relatively resistant to chemo-

radiotherapy and to donor-anti-recipient alloreactivity [35–39]. We did not determine the chimerism of A-IgE plasma cells as this would be technically difficult. Plasma cells are virtually absent in blood. The only liquid organ, from which plasma cells could be FACS-sorted, is marrow. Total (rather than A-IgE) plasma cells would need to be sorted from the marrow to get a sufficient number of plasma cells for the STR chimerism determination. After determining the chimerism of the total plasma cells, it would remain unclear whether the same chimerism applies to A-IgE plasma cells in the marrow or, more important, to A-IgE plasma cells in an atopic disease-pertinent organ like the respiratory mucosa [40]. Persistence of atopy to food allergens post transplant was noted in children undergoing HCT for dedicator of cytokinesis 8 (DOCK8) deficiency. Consistent with the hypothesis of persistence of A-IgE plasma cells, these children showed complete chimerism in blood but incomplete chimerism in marrow nucleated cells [41].

Secondly, we could not ascertain whether unrelated donors were on anti-allergic therapy. Therefore, we cannot exclude the possibility that a donor had A-IgE <0.35 kU_A/L, because of therapy. This could result in a misclassification of a D+R- patient as D-R-, or a D+R+ patient as D-R+. Nevertheless, the former misclassification would not change the conclusion that transfer of atopy appears to be rare. Likewise, the latter misclassification could not change the conclusion that atopy persists after HCT in some D-R+, as four of the five D-R+ patients with persisting atopy had related donors, on whom we knew that they were not treated with desensitization immunotherapy, anti-IgE, or systemic corticosteroids. Even if some of the unrelated donors were on a corticosteroid, it might not have resulted in donor atopic status misclassification, as positivity of skin prick test and thus presumably A-IgE in the atopic range in serum is unlikely to be influenced by corticosteroids [42]. Also, it is very unlikely for an unrelated donor center to allow a person treated with anti-IgE or a corticosteroid to become a donor.

Thirdly, we studied only 54 patients, of whom only 12 were D-R+ and only 11 D+R-. Even though this is the largest study that has been done on atopy cure and transfer, a larger sample size would allow for more definite conclusions on the likelihood of atopy “cure” or transfer and the potential factors influencing the likelihood.

Fourthly, seasonality (differences in exposure to certain allergens between, e.g., winter and summer) was not taken into account.

Fifthly, we focused on atopy and not atopic disease, the latter being clinically relevant. A study focusing on atopic disease is needed. However, our preliminary data on atopic asthma/rhinitis suggest that an adequately powered study on atopic disease cure/transfer will require a lot of resources, as of 69 patients accrued, only 11 were evaluable (proceeded

into related donor transplantation, their donor agreed to participate, atopic asthma/rhinitis pretransplant status could be unequivocally assigned to both the patient and the donor, and the patient survived without relapse, graft failure, new malignancy, or loss to follow up until ≥ 2 years post transplant). Moreover, among the 11 evaluable patients, only five were D-R+ and two were D+R-. Moreover, among the five D-R+ patients, only one had an unequivocal atopic asthma/rhinitis status post transplant (Table 2). Given the very large number of patients required for a conclusive study on atopic disease cure/transfer and the need for their long-term follow up, it is unlikely that results of such a study will be known in near future.

In conclusion, “cure” of atopy with HCT occurs in about half patients, albeit probably not due to the replacement of atopic with nonatopic immune system but possibly due to an immune reset analogous to that observed in some autoimmune disease patients after autologous HCT. Transfer of atopy appears to occur rarely. Future research should focus on whether the same applies to atopic diseases like atopic asthma/rhinitis/eczema and, if yes, on elucidating the factors influencing whether the cure or the transfer occurs.

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Author contributions Contribution: SW analyzed the data and wrote the manuscript. AC supervised the A-IgE determination. GT, RD, and AA performed chimerism analysis. PD-K and AK enumerated CD4 T cells. MM, PK, PS, MNG, VL, MM, LL, AD, TVS, JB, and SS facilitated patient accrual and collection of specimens. RL and FMK provided intellectual input. JS designed the study, supervised the analyses and edited the manuscript.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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Supplementary Methods

Patients and Transplantation

Patients from two sources were studied: 1. patients participating in a prospective study of the transferability/curability of atopic asthma/rhinitis (both symptoms of asthma/rhinitis and serum A-IgE levels were evaluated), and 2. patients participating in a laboratory study of immunity (only serum A-IgE levels were evaluated).

A total of 69 patients likely to need allogeneic hematopoietic cell transplantation agreed to participate in the prospective study of atopic asthma/rhinitis transferability and curability. The patients and their donors filled out a questionnaire on symptoms of asthma and rhinitis (see the end of Supplementary Material online) and had pretransplant blood collected and serum stored for allergen-specific IgE (A-IgE) level determination. This was repeated in the patients annually posttransplant starting at 2 years. In patients treated with chemotherapy before HCT (eg, for acute leukemia), the pretransplant blood was collected before chemotherapy, as A-IgE level in an atopic person might transiently drop to the nonatopic range after induction/consolidation chemotherapy, which could result in classifying an atopic recipient as nonatopic.¹⁸ Similarly, patients treated with tyrosine kinase inhibitors (TKI, eg, imatinib, dasatinib, nilotinib) before HCT were not eligible, as TKIs are known to be immunosuppressive.¹⁹⁻²¹ Of the 69 patients, only 11 were evaluable for their atopic asthma/rhinitis status after HCT, i.e., proceeded into related donor transplantation (consenting unrelated donors for filling out questionnaires was considered unfeasible), their donor agreed to participate, atopic asthma/rhinitis pretransplant status could be unequivocally assigned to both the patient and the donor (positive if asthma or rhinitis symptoms were present AND A-IgE serum level was ≥ 0.35 kU_A/L, negative if asthma and rhinitis symptoms were absent AND A-IgE was A-IgE < 0.35 kU_A/L, and equivocal if symptoms were present with A-IgE < 0.35 kU_A/L or symptoms were absent with A-IgE ≥ 0.35 kU_A/L), and the patient survived without relapse, graft failure, new malignancy, or loss to follow up until ≥ 2 years post-transplant. The rationale for evaluating atopic asthma/rhinitis status at ≥ 2 years was that pharmacologic immunosuppression was typically discontinued by 1 y, thus exposure to allergens in the second or subsequent years could lead to unopposed activation and

differentiation of allergen-specific B cells into A-IgE plasma cells. HCT dates of the 11 patients were between May 18, 2005 and December 2, 2016. The patients underwent HCT in Calgary or Prague.

Additional 548 patients (of 764 who underwent first allogeneic HCT in Calgary between May 1, 2005 and December 31, 2016) agreed to participate in the study of immunity which included the collection of blood specimens but no symptom questionnaires. A complete set of sera (donor pre-HCT, recipient pre-HCT, and recipient ≥ 2 years post-HCT) was available on 90 patients who survived without relapse, graft failure or second malignancy until ≥ 2 years. Of these, 51 patients were excluded because their pretransplant blood was drawn after chemotherapy or immunosuppressive therapy or on/after TKI treatment and their A-IgE was < 0.35 kU_A/L (patients whose pretransplant blood was drawn after chemotherapy or TKI but contained ≥ 0.35 kU_A/L A-IgE were included). This left 39 patients included.

Thus, into the current study of atopy, we included 15 patients from the above prospective study on atopic asthma/rhinitis (11 patients evaluable for atopic asthma/rhinitis status after HCT and 4 patients whose atopic asthma/rhinitis status pretransplant was equivocal (symptomatic patient with A-IgE < 0.35 kU_A/L or asymptomatic patient with A-IgE ≥ 0.35 kU_A/L) but who otherwise fulfilled the criteria in the above paragraph) plus the additional 39 patients, i.e., a total of 54 patients. Their demographic and clinical characteristics are displayed in Table 1.

None of the 54 patients and none of their related donors was treated in the pretransplant year for allergy with immunosuppressive drugs like corticosteroids, desensitization immunotherapy or anti-IgE. This was not known for unrelated donors. No patients developed graft failure. After transplant, some D-R+, D+R-, and D+R+ patients were treated with antihistamines or topical anti-asthma/rhinitis drugs, but no patient was treated with desensitization immunotherapy or with an anti-IgE or a T or B cell antibody. Seven patients developed relapse of their hematologic malignancy or a new malignancy (discounting nonmelanoma skin cancer or carcinoma in situ) at ≥ 2 years posttransplant. Their follow up was terminated at the last annual evaluation for atopy before the diagnosis of relapse or new malignancy.

Four autologous HCT recipients, atopic pre-HCT, were also studied. These were derived from 48 patients who underwent autologous HCT for lymphoma, whose pretransplant serum was available, and who

survived without relapse or second malignancy until ≥ 2 years posttransplant. Of the 48 patients, only 4 had pre-HCT A-IgE ≥ 0.35 kUA/L and donated blood at ≥ 2 years post-HCT (median 5 years, range 2-12 years). Their median age at transplant was 50 years (range, 39-70). Conditioning was with rituximab + gemcitabine + melphalan for 2 patients, rituximab + melphalan + total body irradiation for 1 patient, and with rituximab + carmustine + etoposide + cytarabine + melphalan for 1 patient. All 4 patients were in remission (of lymphoma) and not treated with antineoplastic therapy since the autologous HCT.

Twelve transplant donors were studied for A-IgE levels not only pre-HCT but also at ≥ 2 years post-HCT. Their median age was 52 years (range, 21-67).

Determination of multiallergen- or single allergen-specific IgE

Multiallergen-specific IgE (in this paper abbreviated as “A-IgE”) serum levels were determined using the ImmunoCAP Phadiatop test (Thermo Fisher Scientific Inc., Uppsala, Sweden). This test measures IgE against eleven common aeroallergens, specifically, allergens extracted from dust mites (*Dermatophagoides farinae* and *Dermatophagoides pteronyssinus*), cat dander, dog dander, horse dander, timothy grass pollen, mugwort pollen, pellitory-of-the-wall (upright pellitory) pollen, birch tree pollen, olive tree pollen, and *Cladosporium herbarum* mold. The solid phase of ImmunoCAP is covalently coupled to standardized allergen source material, recombinant allergen components, or relevant markers respective to the allergen of interest. This solid phase also consists of an optimal amount of cellulose to ensure binding of all relevant IgE antibodies in the patient’s sample. IgE in the serum samples bind to the allergens on the solid phase. After washing out non-specific IgE, mouse anti-human IgE (secondary antibody) bound to an enzyme is added to form a complex and then an enzyme substrate is added, resulting in a fluorescent signal. A-IgE concentration is determined using a standard curve, created by dilution of a known concentration of purified A-IgE over a range of concentrations. Importantly, per expert consensus, the development of which was sponsored by the National Institutes of Health (NIH), multiallergen-specific IgE is considered a core biomarker of atopic disease that permits the characterization of atopic status of patients in prospective clinical trials and observational studies.¹

Single allergen-specific IgE (hereafter “single A-IgE”) was determined using ImmunoCAP assay for single allergens (Thermo Fisher Scientific Inc., Uppsala, Sweden) according to manufacturer’s recommendations.

For both A-IgE (specific for the eleven allergens) and single A-IgE, we used 0.35 kU_A/L as the manufacturer- as well as expert panel-recommended cutoff for designating a patient as atopic vs nonatopic.¹ For the determination of whether a patient was atopic after transplant if A-IgE was measured at multiple posttransplant time points, the last measurement result was used.

B and T cell chimerism determination

Chimerism of T and B cells was determined using cryopreserved blood mononuclear cells (MNC) collected at 1 year after HCT. The reason for determining the chimerism at 1 year was that we wished to know whether by 1 year the T and B cells were from the atopic or the nonatopic donor or recipient, thus whether in the second and subsequent years the donor cells, the recipient cells, or both were exposed to allergens. The MNCs were stained with CD3 antibody conjugated to phycoerythrin (PE, Thermo Fisher, clone SK7) and CD19 antibody conjugated to Brilliant Violet 421 (BV421, BioLegend, clone HIB19). B cells (CD20⁺) and T cells (CD3⁺) were sorted using FACS Aria (BD Biosciences, Mississauga, Canada). DNA was extracted using the QIAamp DNA Micro Kit (QIAGEN Inc., Hilden, Germany) according to the procedure recommended by the manufacturer. The donor/recipient chimerism was determined using Identifiler STR (short tandem repeat) panel that includes 16 STR markers on 14 chromosomes (AmpFI STR Identifiler, Applied Biosystems, Thermo Fisher).²³ The markers were amplified in multiplex PCR using manufacturer-recommended procedure. The PCR products were analyzed on ABI-3130 genetic analyzer, using ABI Data Collector and ABI Gene Mapper software. The chimerism analysis and data interpretation were performed according to published guidelines.²² In a mixing experiment we determined that the detection limit for recipient cells in a mixture of donor and recipient cells is $\leq 1\%$.

CD4 T cell enumeration

Cryopreserved blood mononuclear cells were thawed and stained with CD4 and CD3 antibodies conjugated to fluorochromes, specifically CD4-allophycocyanin (APC, Thermo Fisher, clone RPA-T4) and CD3-phycoerythrin (Thermo Fisher, clone SK7). Percentage of CD4 T cells among lymphocytes (forward scatter low and side scatter low cells) was determined by flow cytometry. Absolute count of CD4 T cells was determined as the percentage x the absolute lymphocyte count (determined by clinical hematology lab) / 100.

Ethics and Data statement

All subjects provided their written informed consent. Procedures were performed in accordance with the Declaration of Helsinki and were approved by the Conjoint Health Research Ethics Board of the University of Calgary (file number REB15-0128 and REB15-1143). S.W. and J.S. analyzed the data, and all other coauthors had access to the primary study data. Deidentified individual participant data are available from J.S. (jstorek@ucalgary.ca) upon request.

Asthma/rhinitis symptom questionnaire

The assessment of symptoms of asthma and rhinitis was based on the validated questionnaires used in the International Study of Asthma and Allergies in Childhood (ISAAC) (Asher M.I. et al: Eur Respir J 1995, 8:483-91). Asthma symptoms were considered present if the patient answered Yes to at least one of the following questions:

- Have you had wheezing or whistling in the chest in the last 12 months?
- In the last 12 months, has your chest sounded wheezy during or after exercise?
- In the last 12 months, have you had a dry cough at night, apart from a cough associated with a cold or chest infection?

Rhinitis symptoms were considered present if the patient answered Yes to the following question:

- In the last 12 months, have you had a problem with sneezing, or a runny, or blocked nose when you DID NOT have a cold or the flu?

For pretransplant patients who were filling out the questionnaire after they had received chemotherapy (eg, induction chemotherapy for acute leukemia), the clause “in the last 12 months” was changed to “in the 12 months before starting chemotherapy”. The reason for this modification was that atopic disease can transiently abate/improve after chemotherapy (Jones P.D. et al: J Paediatr Child Health 1999, 35:269-271; Weigel S. et al: Pediatr Hematol Oncol 2000, 17:659-665).

Asthma/rhinitis severity score

The assessment of asthma/rhinitis severity was based on the Global Initiative for Asthma (GINA) scoring (www.Ginasthma.com, accessed in 2004), as follows:

Rhinitis

None.

0. *None.*

1. *Mild.* Occasional symptoms, no interference with work/school/other activities, no treatment.
2. *Moderate.* Frequent symptoms and interference with work/school/other activities but less than 2 weeks of missed work/school due to rhinitis in the last year, OR any treatment for rhinitis.
3. *Severe.* As moderate, but more than 2 weeks of missed work/school due to rhinitis in the last year is required.

Asthma

0. *None.*

1. *Mild (intermittent).* All of the following criteria have to be present: intermittent symptoms <1 time a week, brief exacerbations (from a few hours to a few days), night time asthma symptoms <2 times a month, normal or unknown lung function between exacerbations, and no asthma therapy except for inhaled β_2 agonist used occasionally.
2. *Moderate.* Severity between mild intermittent and severe (mild persistent or moderate persistent symptoms per GINA severity scale, OR need for any asthma medication around the clock, except for systemic steroids).
3. *Severe.* Continuous symptoms (substantially more than once a day, frequent exacerbations, frequent night-time asthma symptoms affecting sleep (substantially more than once a week), physical activities limited by asthma symptoms, $FEV_1 \leq 60\%$ predicted, OR any of the following: need for systemic corticosteroids for the control of asthma at least once in the last year, near-fatal episode (loss of consciousness or intubation) due to asthma in the last year, hospitalization or visit to the emergency room for asthma in the last year.

For both Rhinitis and Asthma, the scoring was based on the last 12 months (before each time point).

Supplementary Results

Transfer of Atopy in Only 1 of 11 Patients – Potential Reasons

This unexpected result was likely not due to long-term IST, GVHD, or low CD4 T cell counts. The only D+R- patient who became atopic was on IST until over 2 years posttransplant, whereas all 5 D+R- patients who discontinued IST within one year posttransplant remained nonatopic (Fig. 2, *top*). Regarding GVHD, 5 of the 10 patients (50%) who remained nonatopic had no significant GVHD whereas the one patient who became atopic had significant GVHD (Fig. S5). Regarding whether CD4 T cell lymphopenia could explain the lack of atopy after transplant, this is less clear. On one hand the one patient who became atopic had at 2 years the highest CD4 T cell count (of 9 D+R- patients on whom MNCs were available) (Fig. S6). On the other hand, there were two non-GVHD/<1 year IST patients who had a similar CD4 T cell counts (as the patient who became atopic) and these two patients remained nonatopic (Fig. S6). Thus, D+R- patients can remain nonatopic even in the absence of long-term pharmacologic immunosuppression, significant GVHD, or marked CD4 T lymphopenia.

To assess whether the persistence of recipient T or B cells accounted for the unexpected lack of atopy transfer, we measured the T and B cell chimerism in 10 (of the 11) D+R- patients whose MNCs from 1 year were available. In the one patient who became atopic, 100% T and B cells were donor. Of the 10 patients who remained nonatopic, MNCs were available on 9 patients. Of these, 8 patients had 100% donor T cells and 100% donor B cells, except for one in whom B cell chimerism could not be determined due to insufficient quantity of B cells, whereas 1 patient had 33% donor T cells with 100% donor B cells (red line in Fig. 2, *top*). In the patient whose MNCs were not available, chimerism among total white blood cells had been determined by a clinical lab at 4 years posttransplant and showed 100% donor cells. Collectively, as at least 8 of the 10 patients who remained nonatopic were by 1 year complete chimeras, persistence of recipient T or B cells cannot explain the lack of the atopy transfer.

Next, we evaluated whether the nonatopic-range A-IgE levels in the 10 patients who remained nonatopic could be due to the lack of posttransplant exposure of the recipient to an allergen(s) to which the donor was atopic. For example, if the only allergen to which the donor was atopic was olive tree pollen, the recipient's IgE

specific for olive tree pollen would not be elevated after transplant due to the absence of olive trees in Alberta. In the sera from the donors of the 10 patients who unexpectedly remained nonatopic, we measured IgE specific for each of the eleven allergens included in the Phadiatop assay. For only 1 of the 10 patients the donor was atopic to only allergens that the recipient may have not been exposed to after transplant (patient No. 1271 in Table S3 whose donor was atopic only to cat and dog dander). We contacted this patient and learnt that he was exposed to cats and dogs on multiple occasions in the second and subsequent posttransplant years. Therefore, in the 10 patients who remained nonatopic, a lack of posttransplant exposure was not the reason.

It is also conceivable that atopy is not transferable and that the posttransplant A-IgE in the atopic range in the one D+R- patient who apparently became atopic was due to falsely negative Phadiatop result in the patient's pretransplant serum (the patient being truly D+R+). In the pre-transplant serum, we measured IgE specific for each one of the eleven allergens. The concentration of IgE for each allergen was $<0.35 \text{ kU}_A/\text{L}$ (data not shown). Thus, this patient was correctly classified as D+R-. Thus, true transfer of atopy occurred in the one patient.

Atopy before and after autologous HCT

As atopy abated even in D+R+ patients, we wished to evaluate whether the transplant conditioning itself could have an anti-atopic effect. To assess this, we measured A-IgE at ≥ 2 years posttransplant in 4 autologous HCT recipients who were atopic pretransplant. The A-IgE levels declined in all 4 patients (median 10.2-fold), and 3 of the 4 patients became nonatopic (Fig. S9). This suggests an anti-atopic effect of transplant conditioning. However, the transplant conditioning of all 4 patients contained rituximab. Therefore, it is not clear whether the high dose chemotherapy or the rituximab (or both) resulted in the A-IgE decline.

Reanalysis of the Curability and the Transferability after Exclusion of Patients and Donors with

Borderline A-IgE Pretransplant

As low-positive A-IgE values are only mildly associated with clinical allergic disease,³¹⁻³² we reanalyzed the above data after excluding patients whose A-IgE pre-HCT was 0.35 to 0.70 kU_A/L or whose donor A-IgE was 0.35 to 0.70 kU_A/L. As shown in Fig. S10-12, the above main conclusions remained valid, i.e., that atopy is curable in a substantial fraction of D-R+ patients and surprisingly also D+R+ patients, and that atopy may be transferred rarely. Specifically, of 9 D-R+ patients, 2 remained atopic, 1 became ambiguous (A-IgE 0.35-0.70 kU_A/L), and 6 became nonatopic. Of 8 D+R- patients, none became atopic, 1 became ambiguous, and 7 remained nonatopic. Of 13 D-R- patients, 11 remained nonatopic and 2 became ambiguous. Of 8 D+R+ patients, 2 remained atopic, 2 became ambiguous, and 4 became nonatopic.

Supplementary Tables

Table S1. Single allergen A-IgE levels (kU_A/L) in the donors of the D-R+ patients with persistent atopy after HCT.

Patient*	DF	DP	Cat	Dog	Horse	TG	MW	WP	Birch	Olive	CH
5001 (blue)	0.02	0.02	0.00	0.00	0.00	0.01	0.00	0.02	0.00	0.00	0.00
1467 (black)	0.11	0.08	0.00	0.00	0.00	0.01	0.01	0.02	0.01	0.02	0.01
895 (yellow)	0.03	0.02	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.04
929 (green)	0.01	0.00	0.00	0.00	0.00	0.00	0.21	0.01	0.00	0.01	0.00
867 (grey)	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

* Unique patient number (line color in Fig. 1).

Allergens: DF = Dermatophagoides farinae, DP = Dermatophagoides pteronyssinus, Cat = cat dander, Dog = dog dander, Horse = horse dander, TG = Timothy grass pollen, MW = Mugwort pollen, WP = wall pellitory pollen, Birch = birch tree pollen, Olive = olive tree pollen, CH = Cladosporium herbarum.

Table S2. Single allergen A-IgE levels (kU_A/L) in the D+R- patient who became atopic after HCT,* and in his donor pretransplant.

Time point	DF	DP	Cat	Dog	Horse	TG	MW	WP	Birch	Olive	CH
Donor pre-HCT	0.04	0.02	1.63	0.26	0.01	5.85	0.16	0.42	0.15	0.20	0.02
Recipient pre-HCT	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Recipient at 2 y post-HCT	0.09	0.10	0.04	0.09	0.04	0.29	0.06	0.06	0.04	0.04	0.04
Recipient at 3 y post-HCT	0.07	0.10	0.02	0.06	0.02	0.23	0.03	0.03	0.02	0.03	0.03
Recipient at 7 y post-HCT	0.05	0.04	0.01	0.02	0.00	0.58	0.03	0.01	0.02	NSQ	0.01

* Grey line in Fig. 2, *top*.

Allergens: DF = Dermatophagoides farinae, DP = Dermatophagoides pteronyssinus, Cat = cat dander, Dog = dog dander, Horse = horse dander, TG = Timothy grass pollen, MW = Mugwort pollen, WP = wall pellitory pollen, Birch = birch tree pollen, Olive = olive tree pollen, CH = Cladosporium herbarum.

Table S3. Single allergen A-IgE levels (kU_A/L) in the donors of the D+R- patients who remained nonatopic after HCT.

Patient*	DF	DP	Cat	Dog	Horse	TG	MW	WP	Birch	Olive	CH
1499 (light blue)	15.10	10.00	0.15	0.07	0.60	0.57	0.36	0.02	4.04	0.03	0.11
969 (black)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.87	0.00	0.00
1071 (blue)	34.2	65.6	3.76	0.43	0.05	89.6	1.50	0.58	11.4	2.39	0.01
1270 (yellow)	0.18	0.18	21.90	1.14	1.52	>100	1.22	0.29	86.8	1.88	0.04
1271 (purple)	0.02	0.01	10.60	0.61	0.19	0.32	0.00	0.00	0.00	0.00	0.02
5004 (green)	0.02	0.01	0.06	0.15	0.00	0.57	NSQ	NSQ	44.8	NSQ	0.02
1192 (red)	0.27	0.27	0.01	0.03	0.00	0.00	0.00	0.02	9.38	0.00	0.00
1383 (brown)	0.93	0.42	0.04	0.06	0.01	25.6	0.46	0.35	0.33	0.90	0.00
1460 (orange)	0.00	0.00	0.00	0.00	0.00	4.88	0.00	0.01	0.00	0.03	0.00
1186 (pink)	0.08	4.97	0.04	0.18	0.04	10.7	0.35	0.21	23.1	0.40	0.02

* Unique patient number (line color in Fig. 2).

Allergens: DF = Dermatophagoides farinae, DP = Dermatophagoides pteronyssinus, Cat = cat dander, Dog = dog dander, Horse = horse dander, TG = Timothy grass pollen, MW = Mugwort pollen, WP = wall pellitory pollen, Birch = birch tree pollen, Olive = olive tree pollen, CH = Cladosporium herbarum.

NSQ = not sufficient quantity of serum

Supplementary Figures

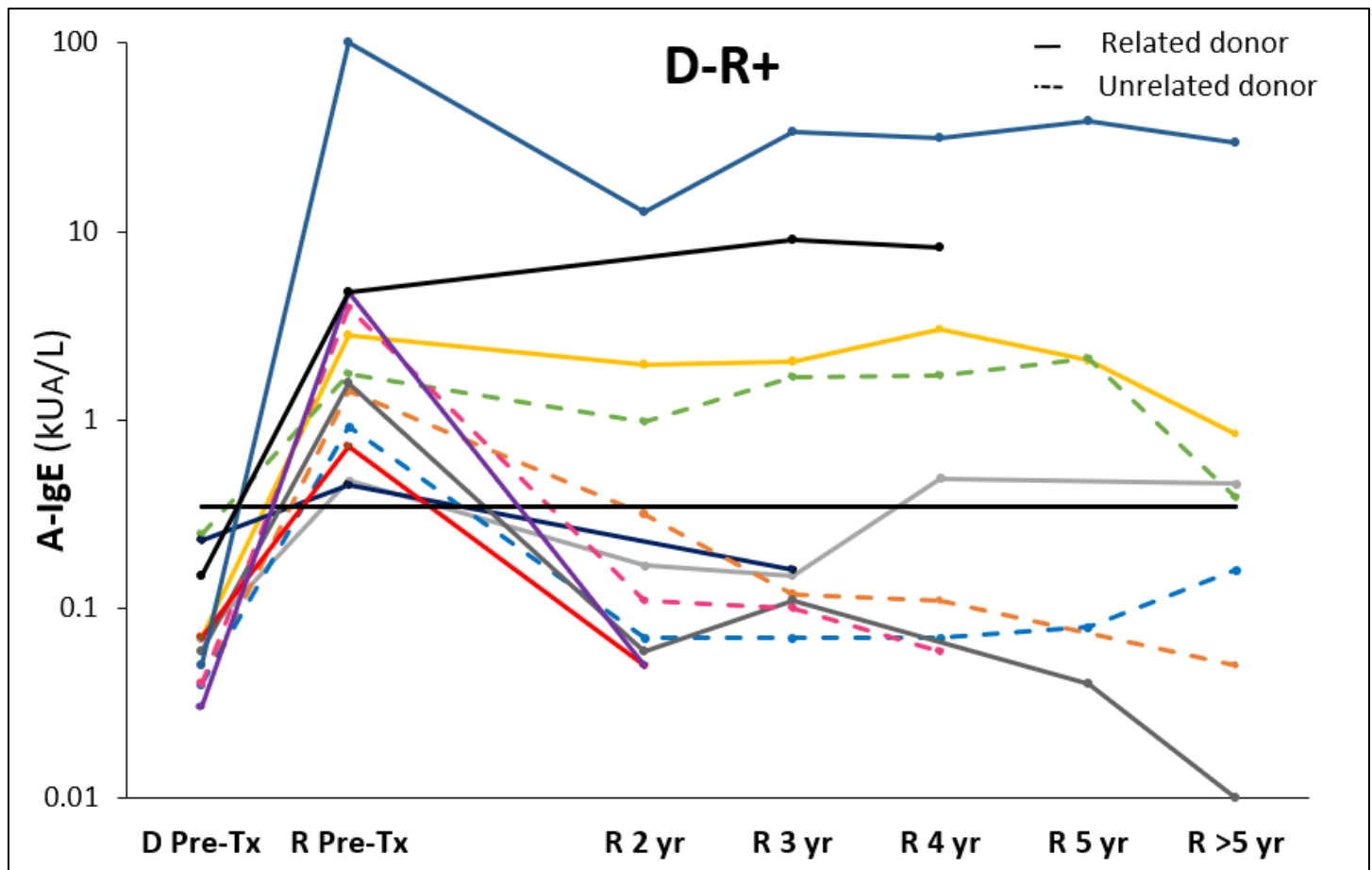


Fig. S1. Allergen-specific IgE (A-IgE) levels in D-R+ patients. On the x-axis, D Pre-Tx denotes donor pretransplant, R Pre-Tx denotes recipient pretransplant, and R X y denotes recipient at 2, 3, 4, 5, and >5 (6-12) years posttransplant. The horizontal line indicates the cutoff (0.35 kUA/L) for atopy. Patients with related donors are indicated with a solid line; patients with unrelated donors are indicated with a dashed line.

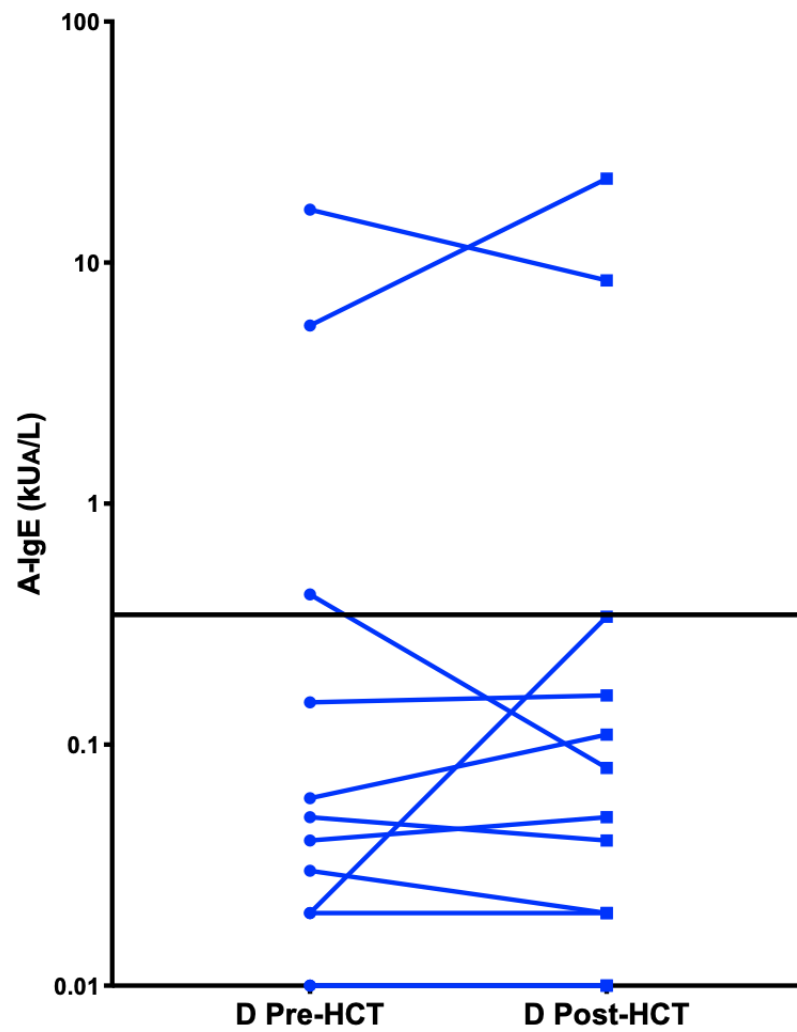


Fig. S2. Allergen-specific (A-IgE) levels before and after HCT in donors. Post-HCT A-IgE levels were measured at 3-12 years posttransplant. The horizontal line indicates the cutoff (0.35 kUA/L) for atopy.

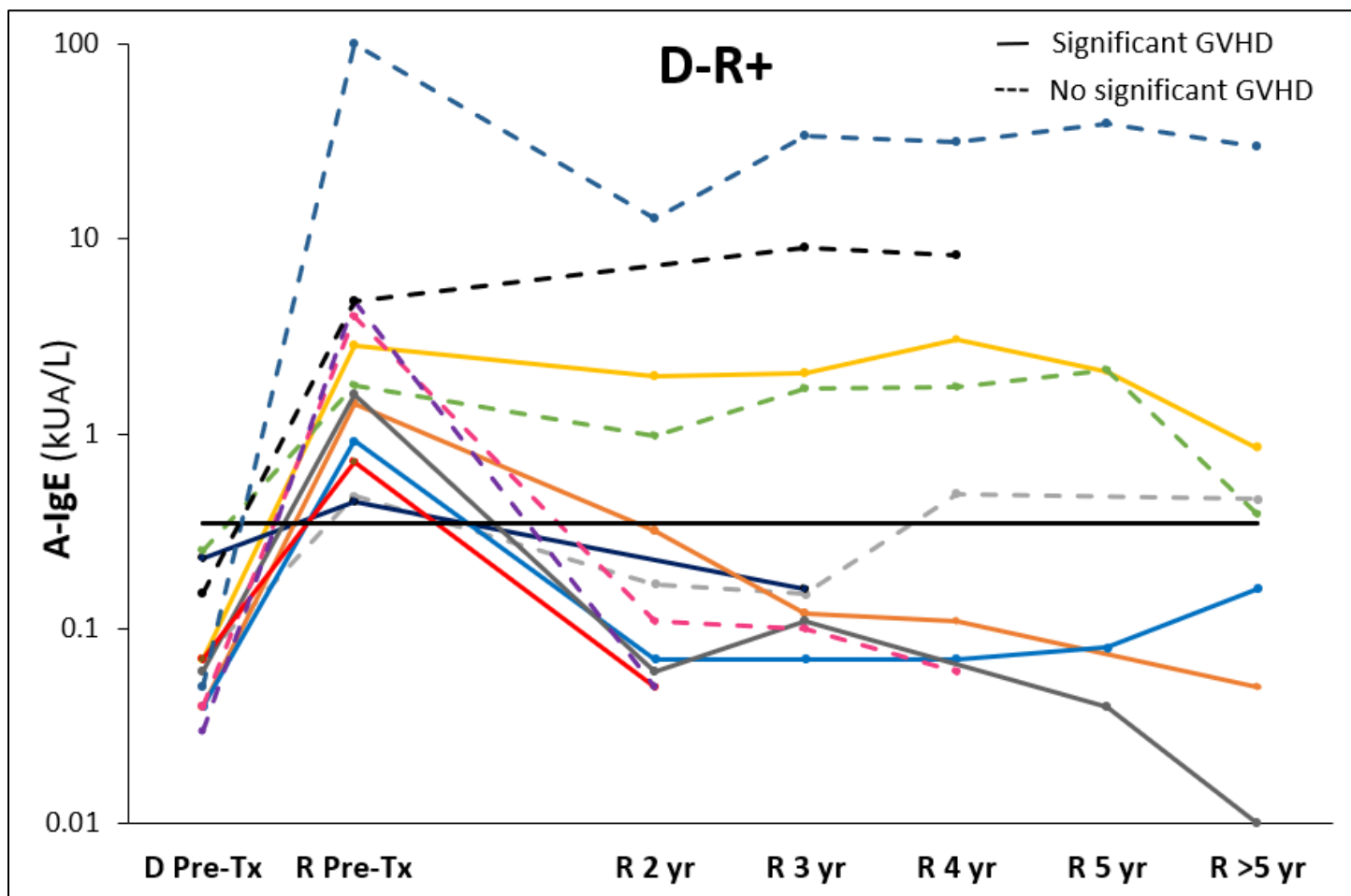


Fig. S3. Allergen-specific IgE (A-IgE) levels before and after HCT in D-R+ patients. On the x-axis, D Pre-Tx denotes donor pretransplant, R Pre-Tx denotes recipient pretransplant, and R X y denotes recipient at 2, 3, 4, 5, and >5 (6-10) years posttransplant. The horizontal line indicates the cutoff (0.35 kU_A/L) for atopy. Patients with significant GVHD are indicated with a solid line; patients with no significant GVHD are indicated with a dashed line.

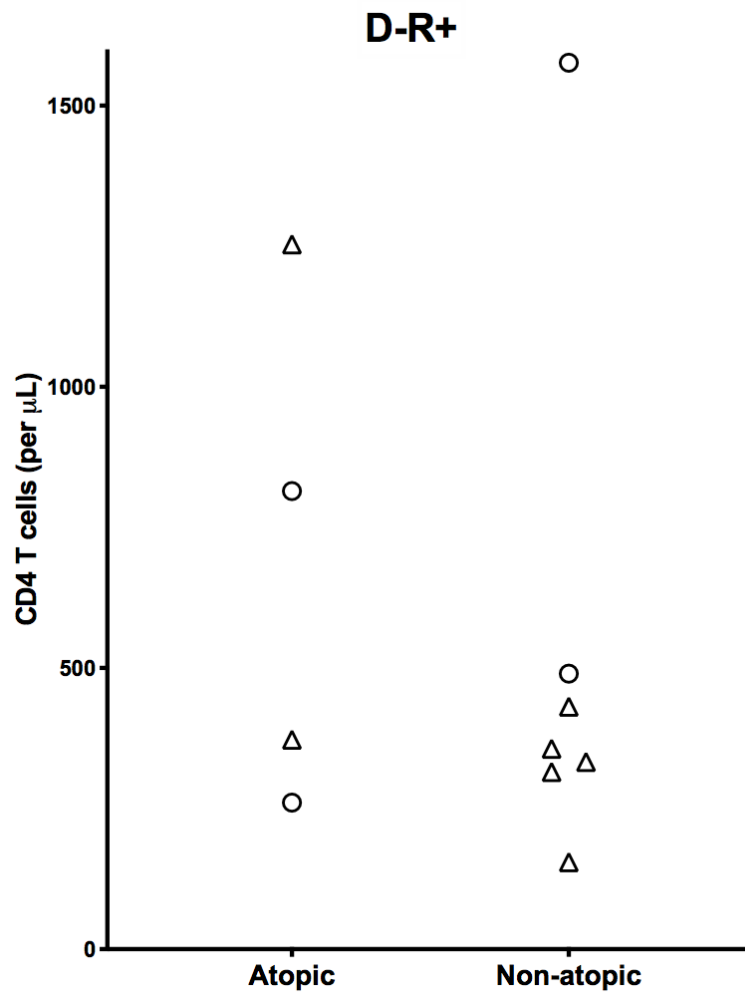


Fig. S4. CD4 T cell counts at 2 years post-HCT in D-R+ patients who after transplant remained atopic (n=4) vs became nonatopic (n=7). Patients who had significant GVHD or were on IST for more than 1 year post-HCT are indicated by a triangle. Among total patients, the median CD4 T cell count in posttransplant atopic vs nonatopic patients was 593.7 vs 356.4/ μL ($p=0.65$, Mann-Wilcoxon test (MWT)). Among patients without significant GVHD and on IST for <1 year, the median CD4 T cell count in posttransplant atopic vs nonatopic patients was 537.8 vs 1033/ μL ($p=0.67$, MWT).

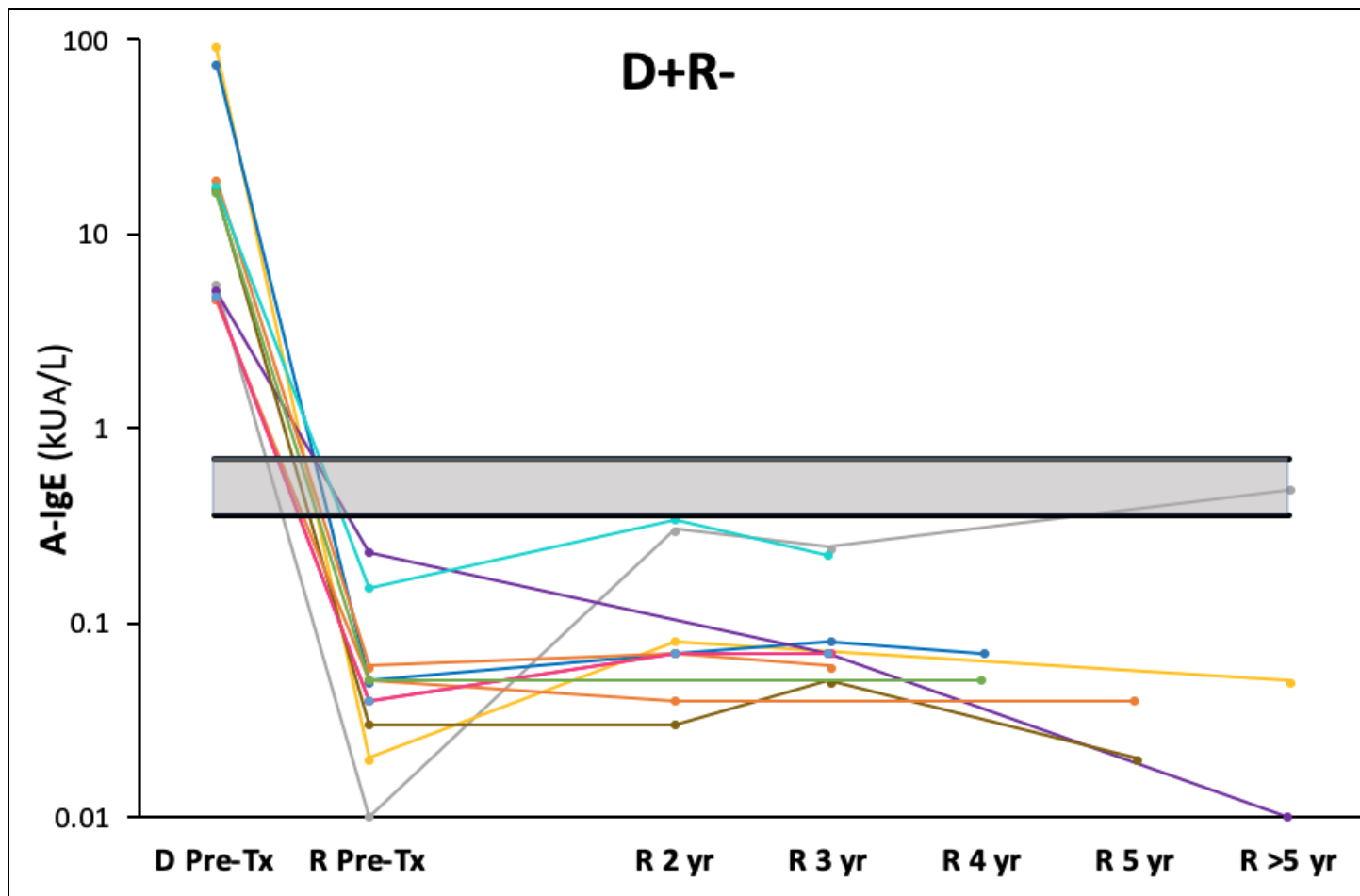


Fig. S5. Allergen-specific (A-IgE) levels before and after HCT in in D+R- patients. Patients with pretransplant A-IgE between 0.35 and 0.70 kUA/L or whose donors had pretransplant A-IgE between 0.35 and 0.70 kUA/L are not included. The gray region denotes A-IgE between 0.35 and 0.70 kUA/L.

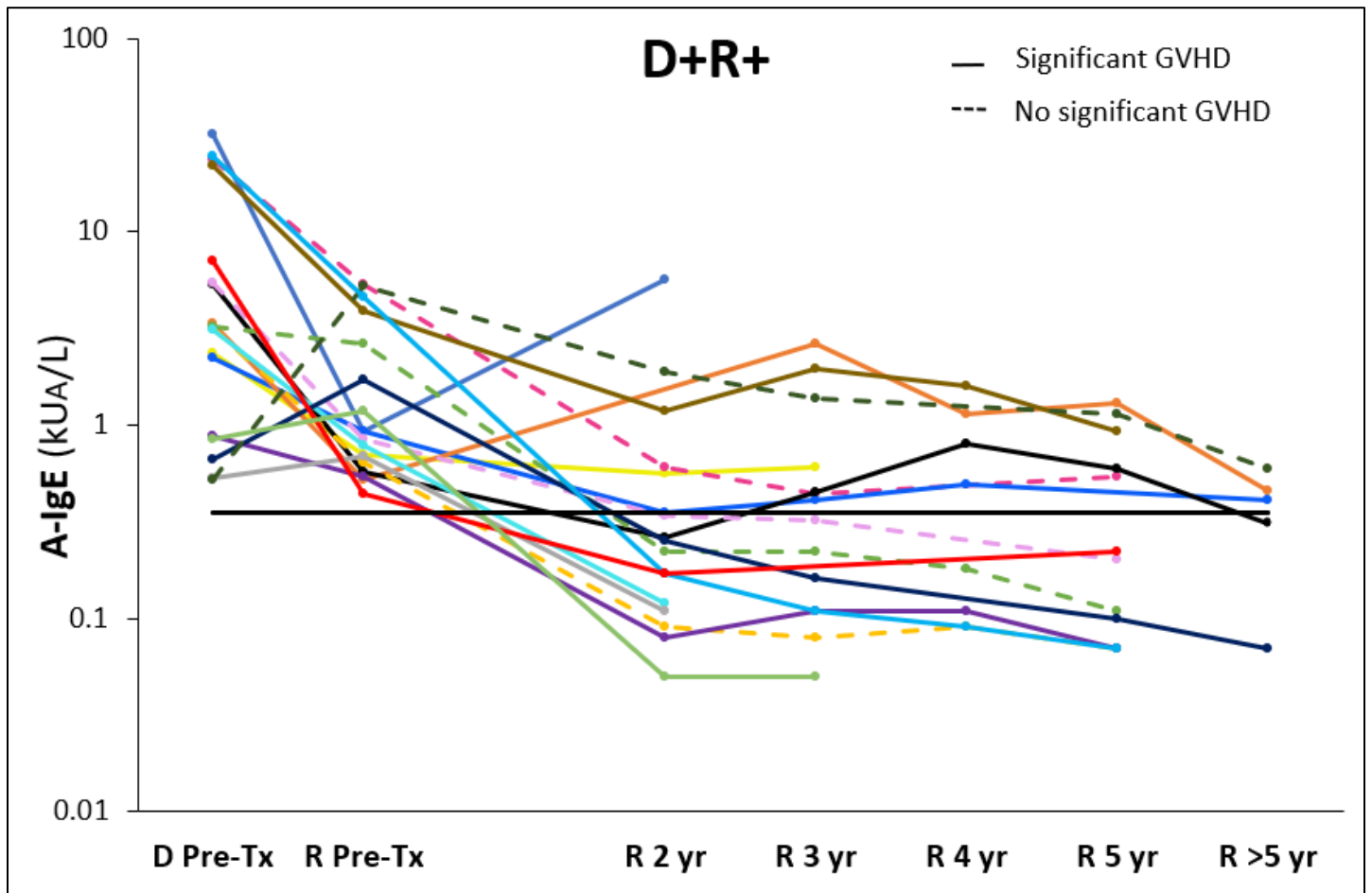


Fig. S6. Allergen-specific IgE (A-IgE) levels before and after HCT in D+R+ patients. On the x-axis, D Pre-Tx denotes donor pretransplant, R Pre-Tx denotes recipient pretransplant, and R X y denotes recipient at 2, 3, 4, 5, and >5 (6-10) years posttransplant. The horizontal line indicates the cutoff (0.35 kUA/L) for atopy. Patients with significant GVHD are indicated with a solid line; patients with no significant GVHD are indicated with a dashed line.

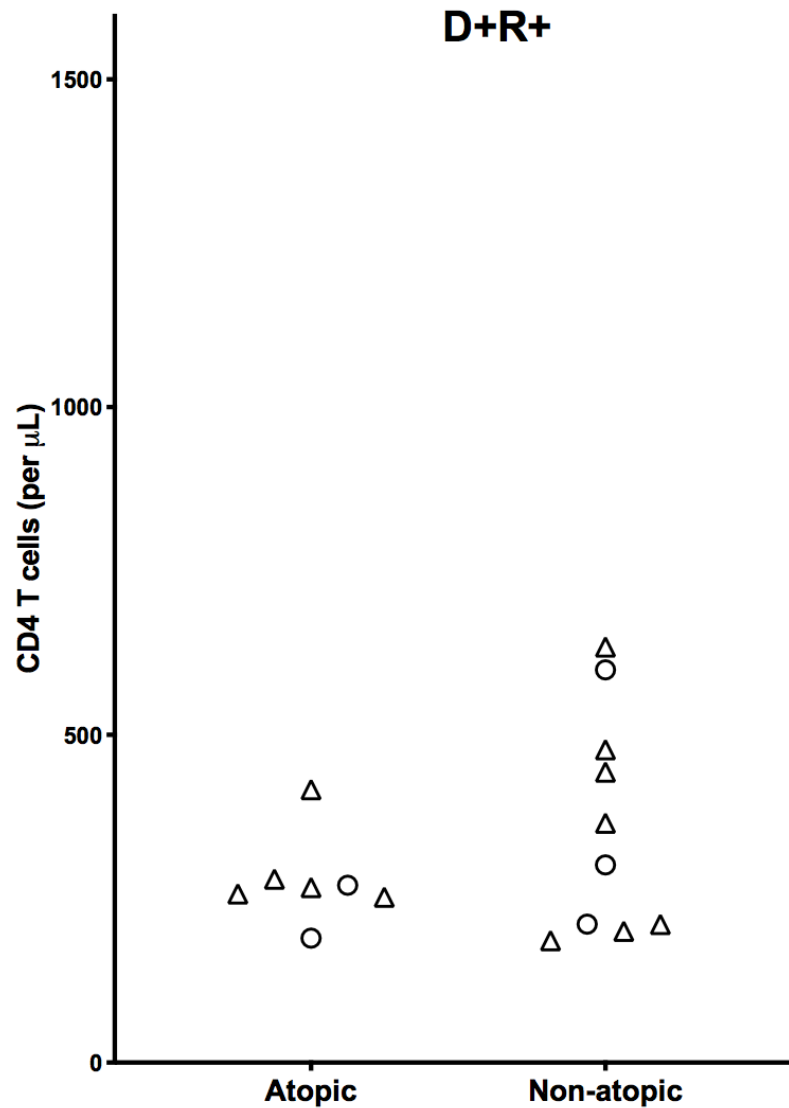


Fig. S7. CD4 T cell counts at 2 years post-HCT in D+R+ patients who after transplant remained atopic (n=7) vs became nonatopic (n=10). Patients who had significant GVHD or were on IST for more than 1 year post-HCT are indicated by a triangle. Among total patients, the median CD4 T cell count in posttransplant atopic vs nonatopic patients was 267 vs 333/ μL ($p=0.464$). Among patients without significant GVHD and on IST for <1 year, the median CD4 T cell count in posttransplant nonatopic patients was 345/ μL .

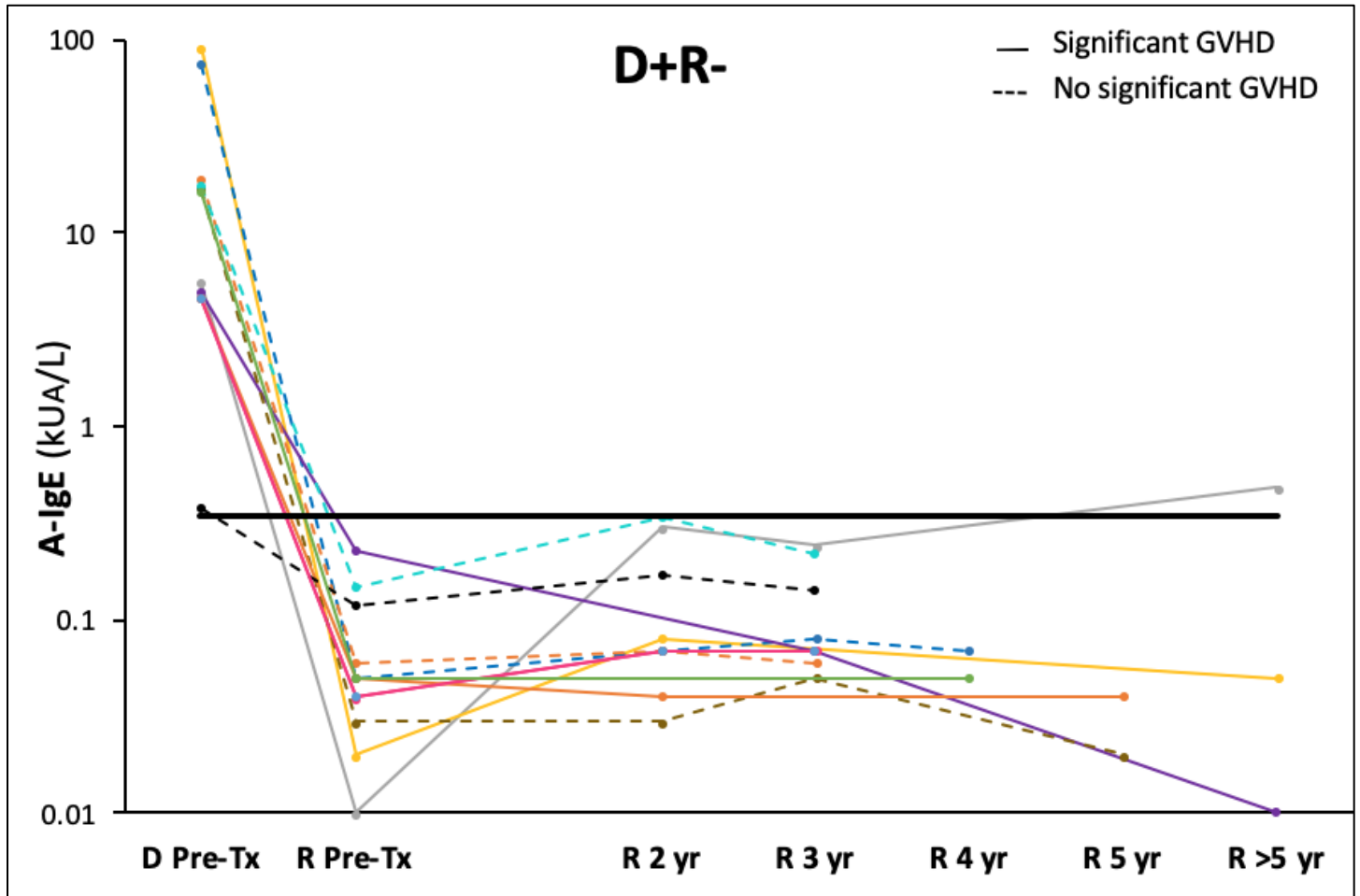


Fig. S8. Allergen-specific IgE (A-IgE) levels before and after HCT in D+R- patients. On the x-axis, D Pre-Tx denotes donor pretransplant, R Pre-Tx denotes recipient pretransplant, and R X y denotes recipient at 2, 3, 4, 5, and >5 (6-12) years posttransplant. The horizontal line indicates the cutoff (0.35 kUA/L) for atopy. Patients with significant GVHD are indicated with a solid line; patients with no significant GVHD are indicated with a dashed line.

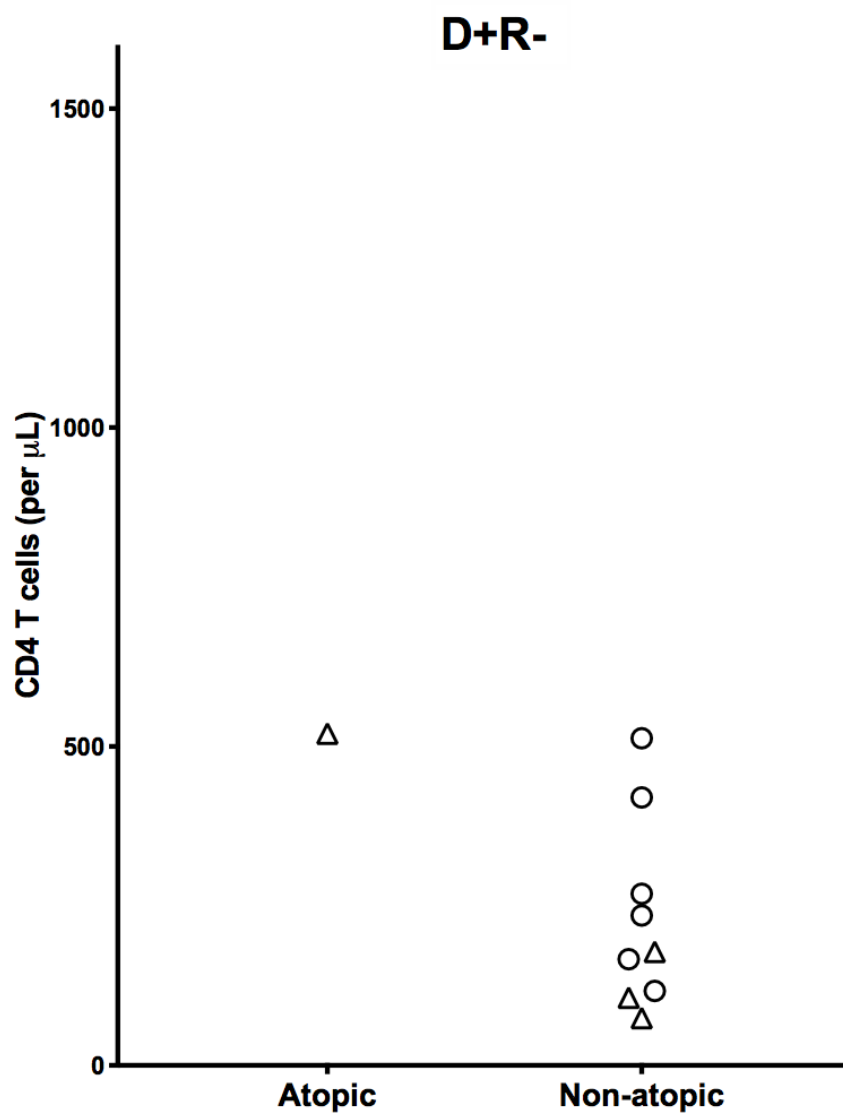


Fig. S9. CD4 T cell counts at 2 years post-HCT in D+R- patients who after transplant became atopic (n=1) vs remained nonatopic (n=9). Patients who had significant GVHD or were on IST for more than 1 year post-HCT are indicated by a triangle. The median CD4 T cell count in posttransplant atopic vs nonatopic patients was 520 vs 178.1/ μL ($p=0.16$).

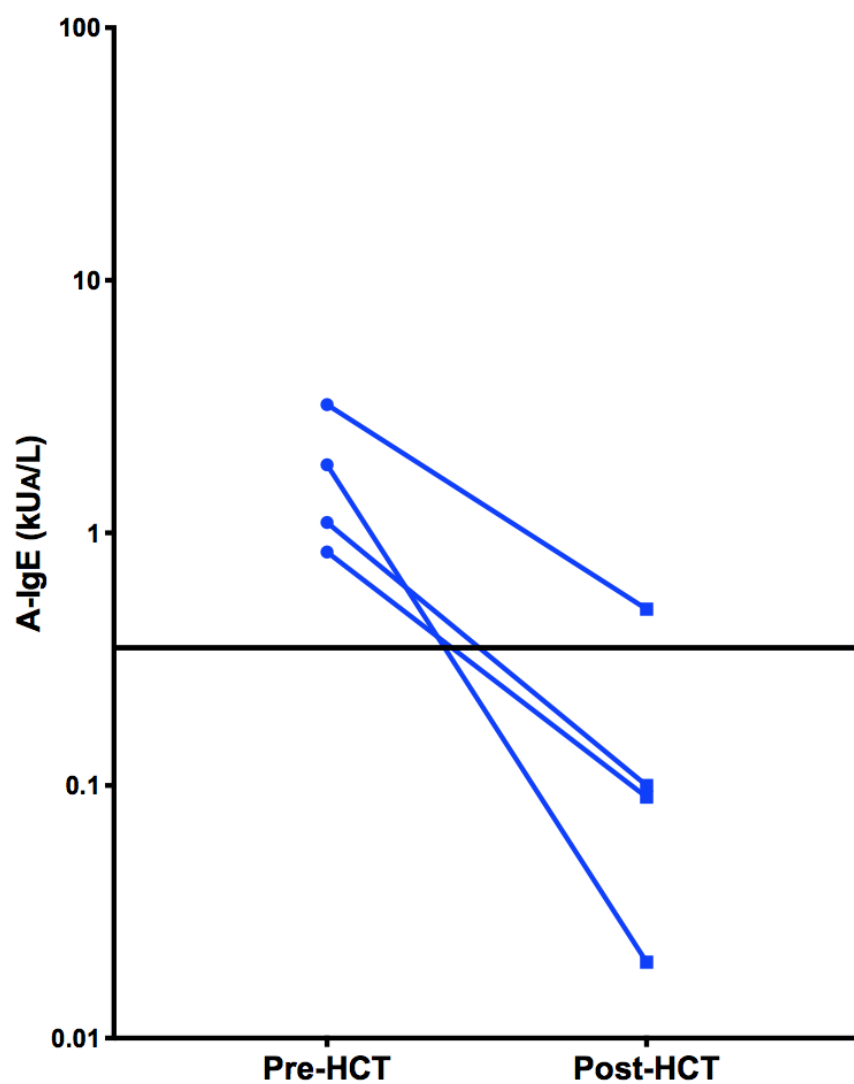


Fig. S10. Allergen-specific IgE (A-IgE) levels before and after autologous HCT (n=4). The horizontal line indicates the cutoff (0.35 kU_A/L) for atopy.

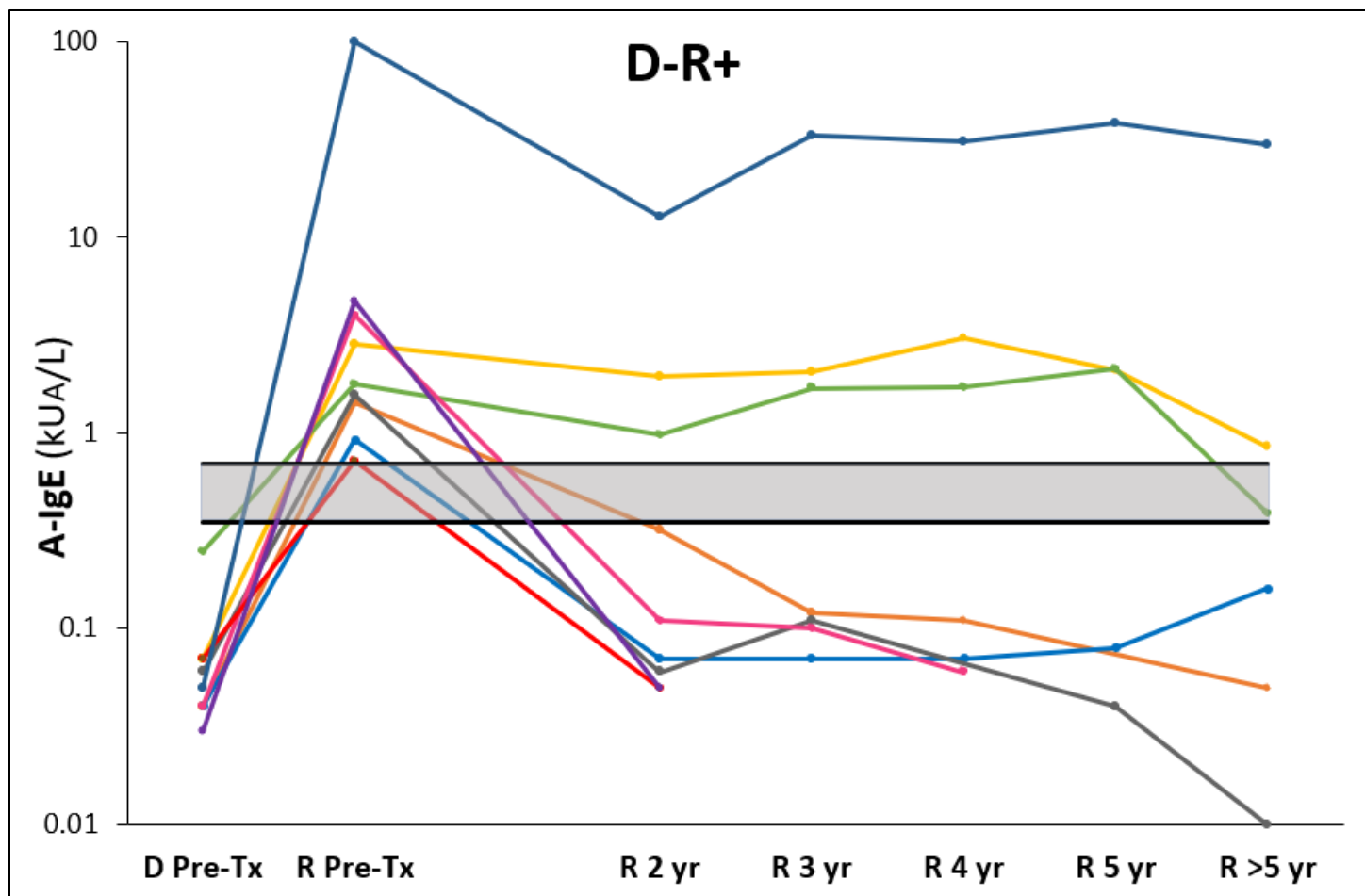


Fig. S11. Allergen-specific (A-IgE) levels before and after HCT in in D-R+ patients. Patients with pretransplant A-IgE between 0.35 and 0.70 kUA/L or whose donors had pretransplant A-IgE between 0.35 and 0.70 kUA/L are not included. The gray region denotes A-IgE between 0.35 and 0.70 kUA/L.

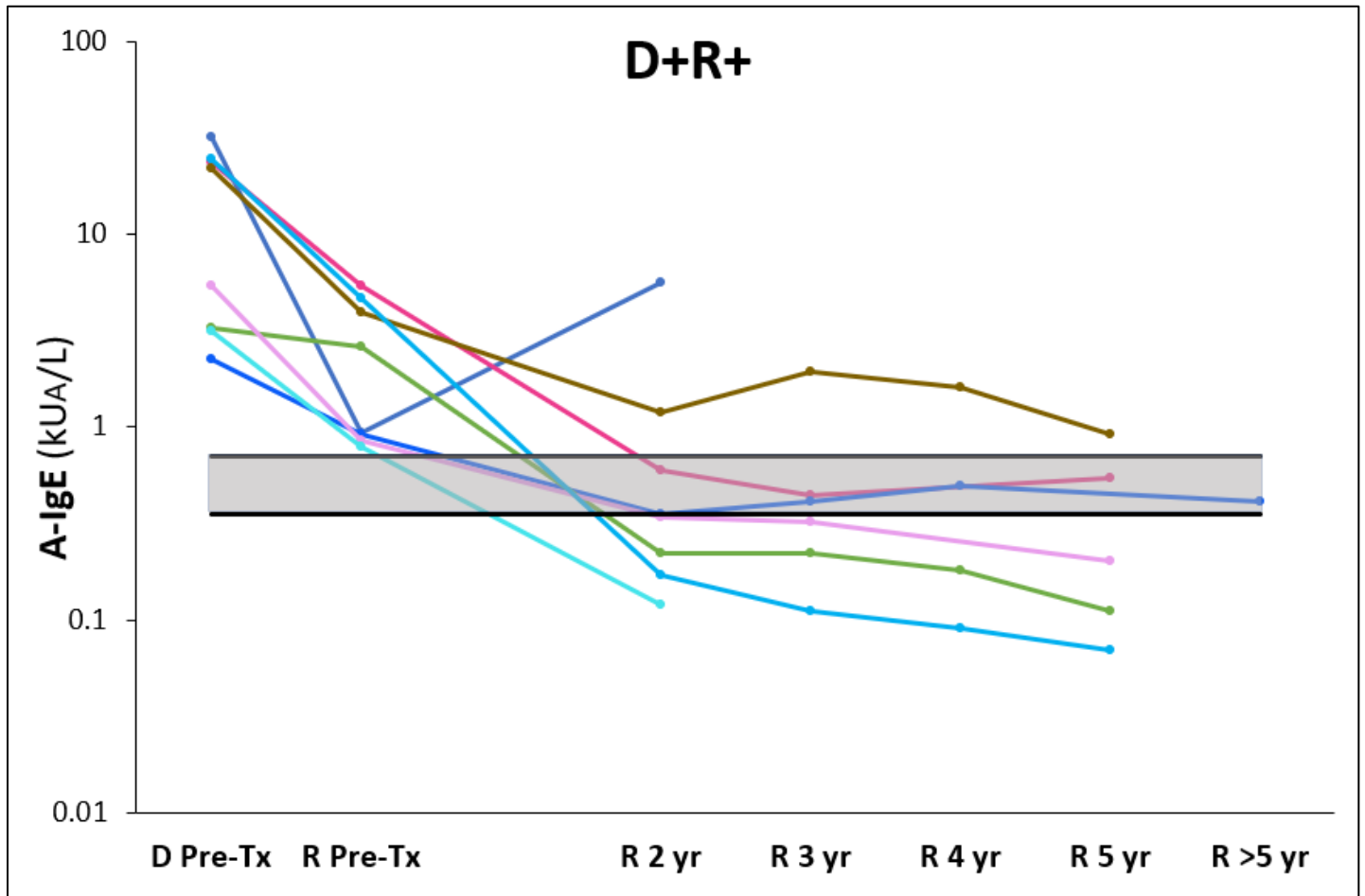


Fig. S12. Allergen-specific (A-IgE) levels before and after HCT in D+R+ patients. Patients with pretransplant A-IgE between 0.35 and 0.70 kUA/L or whose donors had pretransplant A-IgE between 0.35 and 0.70 kUA/L are not included. The gray region denotes A-IgE between 0.35 and 0.70 kUA/L.