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Immune activation- and regulation-related patterns in stable hand transplant recipients

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SUMMARY

We assessed cell subsets and expression of a set of genes related to the T-cell populations in peripheral blood mononuclear cells to elucidate whether immune status of stable hand transplant recipients (HTx) differs from stable kidney transplant recipients (KTx). The study was conducted on five HTx 4.8 ± 1.7 years after transplantation and 30 stable KTx 7.9 ± 2.4 years after transplantation as well as 18 healthy volunteers. The research involved PBMC gene expression analysis of *CD4*, *CD8*, *CTLA4*, *GZMB*, *FOXP3*, *IL10*, *IL4*, *ILR2A*, *NOTCH*, *PDCD1*, *PRF1*, *TGF-B*, *and TNF-A* genes on a custom-designed low-density array (TaqMan) as well as flow cytometry assessment of lymphocyte subpopulations. HTx presented significantly increased expression of immunomodulatory genes (*TNF, IL10, GITR*, and *PDCD1*) compared to KTx and controls. HTx revealed a proinflammatory molecular pattern with higher expression of *NOTCH* and *CD8* compared to KTx and controls. KTx showed a reduced level of regulatory T cells compared to controls and HTx. Both HTx and KTx presented an increased number of $CD8^+$ and $CD8^+CD28^-$ T cells compared to controls. Stable hand transplant recipients exhibit persistent immune activation with rejection-related gene expression pattern counterbalanced by secondary induction of regulatory mechanisms.

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gene expression, hand transplantation, immunomodulation, kidney transplantation, rejection, T cells

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Introduction

The long-term outcome of transplantation depends not only on the surgical skills of the transplant surgeon but also on subsequent adaptive immune responses to the grafted tissues. Interaction of donor major histocompatibility complex with recipients T lymphocytes initiates T-cell activation and the subsequent antigraft response. The effector stage of rejection is characterized by upregulation of cell surface receptors, cytokines, adhesion molecules, and apoptosis-related markers. In spite of immunosuppressive therapy, the rate of reversible acute rejection is almost 100% in vascularized composite allotransplantation (VCA) and together with chronic rejection may lead to allograft loss [1,2].

Induction of donor-specific tolerance is a main goal in transplantation. Hand transplantation is not a life-saving or life-extending procedure, so the biological risk

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of long-lasting immunosuppression is widely discussed. Operational tolerance may free transplant recipients from side effects of immunosuppressive therapy, as well as improving long-term results by cessation of acute and chronic rejection. Many studies have been performed to assess the possibility of inducing tolerance in hand transplant recipients so far [3,4].

Naturally occurring regulatory T cells (Tregs) have been recognized as a T-cell subset with immunomodulatory properties. They play an important role in immune regulation after organ transplantation, and they have been proven to prevent allograft rejection.

Long-term stimulation with alloantigens after transplantation leads to conversion of potential effector cells into Tregs with graft-protective properties. The hallmark of regulatory activity of Tregs is the expression of FOXP3 transcription factor but also increased expression of some other genes, which are upregulated in the presence of donor-specific tolerance [5,6].

It is an open question whether immune activation- and regulation-related patterns differentiate stable VCA recipients from stable solid organ transplants and whether lessons from solid organ transplantation may be directly applied to VCA.

The goal of the study was to describe the immune status of stable hand transplant recipients (HTx) in relation to stable kidney recipients (KTx) and healthy volunteers. The gene expression and lymphocyte subpopulations were analyzed in the search for hallmarks of hand transplant acceptance.

Materials

The study was approved by the Bioethical Committee of Wroclaw Medical University and performed in accordance with the World Medical Association Declaration of Helsinki, and the participants provided fully informed consent.

The study was conducted on five hand transplant recipients (HTx; four males, one female, aged 40 ± 11 years at the time of examination), transplanted between 2006 and 2010 in the Subdepartment of Replantation of Limbs, St. Hedwig District Hospital, in Trzebnica. The study group included recipients who were transplanted at the level of the distal forearm (two recipients), the wrist, the upper arm, and bilateral HTx (wrist). All the recipients presented negative T- and B-cell cross-matches before transplantation. HLA mismatches were 5, 5, 3, 5, and 4, respectively. All of the recipients experienced at least one skin rejection episode, treated with corticosteroids and topical tacrolimus [7]. None of the recipients revealed histological signs of skin rejection at the time of evaluation (control biopsies showed Banff grade 0) or 12 months before and after examination. None of the HTx recipients showed presence of DSA (donor-specific antibodies) before and at the moment of examination as we previously described [8].The initial immunosuppressive therapy consisted in all cases of basiliximab, corticosteroids, tacrolimus, and mycophenolate mofetil. The blood samples were collected 4.8 ± 1.7 years after HTx. The immunosuppression at the time of examination is presented in Table 1.

The results were compared to data for 30 stable kidney transplant recipients (KTx) transplanted in the Department of Vascular, General and Transplantation Surgery and followed up in the Department of Nephrology and Transplantation Medicine, Wroclaw Medical University. The samples were chosen from our biobank with best possible match to HTx group (according to clinical and transplant features). They were 21 males and nine females, with the mean age of 37 years, at the time of transplantation. None of the recipients revealed clinical signs of kidney rejection at the time of evaluation. The kidney allograft function was preserved, with the mean serum creatinine concentration 1.25 ± 0.15 mg/dl at the moment of examination. No infection, PTLD/lymphoma/tumor, and rejection were present in the preceding 12 months prior to the study. The blood samples were collected from 4 to 12 years (7.9 ± 2.4) after KTx.

The study also included 18 healthy volunteers (10 males, eight females; age 46 ± 13 years).

Table 1. Maintenance immunosuppressive therapy in hand transplant recipients at the time of examination.

	Recipient no. 1	Recipient no. 2	Recipient no. 3	Recipient no. 4	Recipient no. 5
Prednisone (mg/day)	5	7.5	5	0	5
Mycophenolate mofetil/sodium (mg/day)	1000	720	1000	1000	1000
Tacrolimus (mg/day)	6	5	5	6	9
Tacrolimus (trough level, ng/ml)	11.8	8.2	9.2	8	9.7

The clinical parameters of examined HTx patients and KTx group are described in Table 2.

Methods

Gene expression

Gene expression studies were performed on PBMC isolated from heparinized blood using density gradient centrifugation on Histopaque-1077 (Sigma-Aldrich, Poznan, Poland). RNA was purified from samples of 2×10^6 PBMC with the RNA Blood Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's protocol including genomic DNA removal with RNase-free DNase (Qiagen, Hilden, Germany). The samples were reversely transcribed with the High-Capacity cDNA Reverse Transcription Kit (Life Technologies, Carlsbad, CA, USA). 10 µl of final reaction volume in 100 µl of TaqMan PCR Master Mix was applied to each channel of custom-designed low-density array (TaqMan) and analyzed on a TaqMan 7900HT instrument.

The research involved triplicate analysis of *CD4* (Hs00181217_m1), *CD8* (Hs00233520_m1), *CTLA4* (Hs00175480_m1), *GZMB* (Hs00188051_m1), *FOXP3* (Hs00203958_m1), *IL10* (Hs00174086_m1), *IL4* (Hs00174122_m1), *ILR2A* (Hs00907777_m1), *NOTCH* (Hs01062014_m1), *PDCD1* (Hs01550088_m1), *PRF1* (Hs00169473_m1), *TGFB1* (Hs99999918_m1), *TNF-A* (Hs00174128_m1), and*GITR* (Hs00188346_m1) with two candidate reference sequences: *18S rRNA* (Hs99999901_s1) and *GAPDH* (Hs99999905_m1).

The relative expression data were calculated using 18S rRNA as a reference gene and the healthy volunteer group as a reference group. The expression data were averaged from triple measurement points and are presented as $\Delta\Delta Ct = \text{mean } \Delta Ct_{ref} - \Delta Ct_{sample}$, where $\Delta Ct = Ct_{18S} - Ct_{gene}$, Ct is the cycle threshold value and defines the calculated cycle number, in which the fluorescence measured during PCR reaction increases over the preset threshold value. The relative expression level can be further calculated as $2^{\Delta\Delta Ct}$.

Cell phenotypes

Antibodies

The following mouse anti-human antibodies (Becton Dickinson, Warsaw, Poland) were used for cell phenotyping: anti-CD3-APC (clone UCHT1), anti-CD4-PerCP (clone SK3), anti-CD25-FITC (clone M-A251), anti-

Table 2. Clinical parameters of examined groups.

	HTx 1	HTx 2	HTx 3	HTx 4	HTx 5	Kidney transplant
Recipient gender	4M/1F					21 M/nine F
Age at Tx (mean ± SD), years	36 ± 10					37 ± 14
Donor gender	M	F	M	F	F	16 M/six F/8 no data
Donor age (mean ± SD)	41	53	50	51	47	40 ± 14 years
HLA mismatches	5	5	3	5	4	From 1 to 6, median 4
Panel-reactive antibodies (PRA)	0%	0%	0%	0%	0%	Positive in eight cases
Donor-specific antibodies (DSA)	None	None	None	None	None	None
Cold ischemia time (h)	10	8	9	8	8	25 ± 7 h
Delayed graft function	NA	NA	NA	NA	NA	3
Acute rejection episodes	2	2	2	7	1	Six episodes in six recipients
Maintenance IS	T + M + S	T + M + S	T + M	T + M + S	T + M + S	7 T, 22 C, other 1; 13 M, 15A, other 2; 30 S
Time from Tx to examination	8	6	5	4	4	7.9 ± 2.4 years

T, tacrolimus; C, cyclosporine; M, mycophenolate mofetil; A, azathioprine; S, steroids; IS, immunosuppression.

CD28-PE (clone CD28.2), anti-CD8-FITC (RPA-T8), and anti-CD127-PE (clone hIL-7R-M21).

Whole-blood staining

The samples of whole blood were stained with anti-CD3-APC, anti-CD4-PerCP, anti-CD25-FITC, and anti-CD127-PE for $CD3^+CD4^+CD25^+CD127^{low}$ phenotyping and with anti-CD3-APC, anti-CD8-FITC, and anti-CD28-PE for $CD3^+CD8^+CD28^-$. After incubation, the samples were lysed with BD FACS Lysing Solution, washed with PBS, and subjected to analysis on a FACSCalibur flow cytometer (Becton Dickinson, Warsaw, Poland). The subpopulations were counted in relation to total lymphocytes.

Flow cytometry gating strategy

In case of T, B, and NK, the gating strategy followed the manufacturer protocol for cell counting in the Trucount tubes. To enumerate the T-cell subpopulations, lymphocytes were gated based on forward and side scatter. $CD3^+CD4^+$ cells were selected on CD4 versus CD3 plot. The $CD3^+CD4^+$ cells were further displayed on CD25 versus CD127 plot and $CD25^+CD127^{low}$ gate described Tregs. CD8 CD28 neg cells were calculated from CD8 versus CD28 plot of the $CD3^+$ lymphocyte gate.

The absolute counts were obtained using T/μl from Multitest assay and the subpopulation ratios.

Statistical analysis

Cell populations and gene expression data are presented as mean ± SD for HTx, KTx, and control groups. The HTx group results are also presented in a case-related manner. Gene expression data were statistically analyzed as ΔΔCt that showed normal distribution. The comparisons between the study groups were performed with *t*-test. The significance level of $\alpha = 0.05$, and the Bonferroni–Holm correction for multiple testing was included for cell populations and expression data families [9]. Throughout the text, the adjusted *P*-values are shown. Statistical analysis was performed using the STATISTICA v.10 statistical package (StatSoft, Cracow, Poland).

Results

The detailed cell phenotypes for the hand transplant recipients are presented in Table 3, and the statistical data for study groups are summarized in Table 4.

Standard lymphocyte phenotyping revealed that total T- and NK-cell counts (numbers of cells per microliter) did not differ significantly between studied groups (Table 4). However, in case of both transplant groups, there was a shift to increased number of $CD8^+$ (KTx $P = 0.005$ and HTx $P = 0.035$). HTx presented level of B cells similar to controls, while in case of KTx, the fourfold depletion of B cells was observed ($P < 0.001$).

In the case of hand transplant recipients, the Treg population size ($CD4^+CD25^+CD127^{low}$ absolute count) was similar to the healthy control group. The analysis of kidney transplant recipient group showed reduced size of Treg population compared to controls ($P < 0.001$).

Together with the increased $CD8^+$ population of T lymphocytes, a strong CD28 negativity was observed in HTx with eightfold increase in absolute cell counts compared to controls ($P = 0.003$). Similar phenomenon was observed in KTx with 10-fold increase in absolute cell counts compared to controls ($P < 0.001$).

The gene expression data were obtained for all measurement points except for few *IL4* assays. The

Table 3. Cell phenotypes observed in hand transplant recipients.

Recipient	HTx 1	HTx 2	HTx 3	HTx 4	HTx 5
T lymphocytes/μl	2794	1868	929	1385	2081
T $CD4^+$/μl	745	1200	588	417	1109
T $CD4^+$/T (%)	26.7	64.2	63.3	30.1	53.3
$CD4^+CD25^+CD127^{low}$/μl	41	65	41	29	93
$CD4^+CD25^+CD127^{low}/CD4^+$ (%)	5.5	5.4	7	7	8.4
$CD4^+CD25^+CD127^{low}$/T (%)	1.5	3.5	4.4	2.1	4.5
$CD8^+$/μl	1591	540	312	837	903
$CD8^+$/T (%)	57	28.9	33.6	60.4	43.4
$CD8^+CD28^-$/μl	1402	100	166	617	615
$CD8^+CD28^-/CD8^+$ (%)	88.1	18.6	53.1	73.7	68.1
$CD8^+CD28^-$/T (%)	50.2	5.4	17.8	44.5	29.5
B lymphocytes/μl	347	415	89	64	122
NK cells/μl	576	184	185	229	61

Table 4. Descriptive statistics of cell phenotypes for examined populations.

Cell population	Controls Mean ± SD	KTx Mean ± SD	KTx *P** versus control	HTx Mean ± SD	HTx *P** versus control	*P** HTX versus KTx
T lymphocytes/μl	1342 ± 394	1573 ± 677	0.460	1811 ± 707	0.260	1.000
T $CD4^+$/μl	826 ± 335	618 ± 201	0.087	812 ± 335	0.940	0.550
$CD4^+CD25^+CD127^{low}$/μl	64 ± 30	31 ± 14	**<0.001**	54 ± 26	1.000	0.072
$CD8^+$/μl	435 ± 152	906 ± 528	**0.005**	836 ± 484	**0.035**	1.000
$CD8^+CD28^-$/μl	68 ± 70	738 ± 442	**<0.001**	580 ± 520	**0.003**	1.000
B lymphocytes/μl	234 ± 102	65 ± 62	**<0.001**	207 ± 162	1.000	**0.029**
NK cells/μl	275 ± 75	255 ± 191	0.680	247 ± 194	0.310	0.940

*The significance was tested including Bonferroni–Holm correction at significance level $\alpha = 0.05$. Adjusted *P*-values are shown. The significant *P*-values are shown in bold.

Table 5. Descriptive statistics of examined gene expression.

Gene Symbol	Gene Name	Controls Mean ± SD	KTx Mean ± SD	KTx *P** versus control	HTx Mean ± SD	HTx *P** versus control	*P** HTx versus KTx
CD4	Cluster of differentiation 4	0.00 ± 1.01	0.60 ± 1.02	0.530	0.93 ± 0.32	0.295	0.490
CD8	Cluster of differentiation 8	0.00 ± 1.43	1.03 ± 1.16	0.127	2.95 ± 0.50	**0.003**	**0.011**
CTLA4	Cytotoxic T-cell antigen 4	0.00 ± 1.07	0.05 ± 1.04	1.000	1.44 ± 0.53	0.264	0.396
FOXP3	Forkhead box P3	0.00 ± 0.73	−0.32 ± 0.95	1.000	0.38 ± 0.72	0.320	0.650
GZMB	Granzyme B	0.00 ± 1.94	0.86 ± 1.38	0.729	1.67 ± 1.00	0.324	0.440
IL10	Interleukin 10	0.00 ± 1.46	0.76 ± 1.84	0.980	3.06 ± 2.31	**0.015**	0.162
IL2RA	Interleukin-2 receptor-alpha chain	0.00 ± 0.66	−0.02 ± 0.93	0.940	0.91 ± 1.01	0.168	0.329
IL4	Interleukin 4	0.00 ± 1.00	−0.34 ± 1.12	1.000	1.25 ± 2.84	0.260	0.280
NOTCH1	NOTCH	0.00 ± 0.67	0.23 ± 0.92	1.000	4.25 ± 0.74	**<0.001**	**<0.001**
PDCD1	Programmed cell death protein 1	0.00 ± 0.92	0.06 ± 1.08	1.000	1.53 ± 0.97	**0.038**	0.076
PRF1	Perforin 1	0.00 ± 1.63	0.88 ± 1.29	0.495	1.68 ± 1.01	0.246	0.570
TGFB	Tumor growth factor-beta	0.00 ± 1.38	0.79 ± 1.16	0.520	1.61 ± 0.31	0.152	0.520
TNF	Tumor necrosis factor	0.00 ± 1.17	0.50 ± 0.88	0.800	2.38 ± 1.07	**0.006**	**0.002**
GITR	Glucocorticoid-induced TNFR family-related gene	0.00 ± 1.12	0.58 ± 0.81	0.516	2.33 ± 0.25	**0.002**	**<0.001**

*The significance was tested including Bonferroni–Holm correction at significance level $\alpha = 0.05$. Adjusted *P*-values are shown. The significant *P*-values are shown in bold.

descriptive statistics of expression data are shown in Table 5.

The hand transplant recipients presented significantly higher ΔΔCT values of most examined genes, both regulatory and activatory compared to the KTx and control groups (Fig. 1).

In the HTx group, several genes were upregulated compared to the control group: *CD8* (7.7-fold, $P = 0.003$), *IL10* (8.3-fold, $P = 0.015$), *NOTCH1* (19-fold, $P < 0.001$), *PDCD1* (2.9-fold, $P = 0.038$), *TNF* (5.2-fold, $P = 0.006$), and *GITR* (fivefold, $P = 0.002$).

In the KTx group, no gene expression differed from the control group.

The expression levels also differentiated HTx from KTx group. From the regulatory genes, only *GITR* was significantly upregulated in the HTx group (fivefold, $P < 0.001$).

Also, the rejection-related genes such as *CD8*, *NOTCH*, and *TNF* were expressed at a higher level in the HTx group (3.8-fold, $P = 0.011$; 16.3-fold, $P < 0.001$; 3.7-fold, $P = 0.002$).

Discussion

In our study, we demonstrated for the first time that stable hand transplant recipients (HTx) differ from stable kidney transplant recipients (KTx) in relation to gene expression and T-cell subsets. Rejection-related molecular pattern (expression of *TNF*, *NOTCH*, and *CD8*) was increased in HTx compared to KTx and healthy controls.

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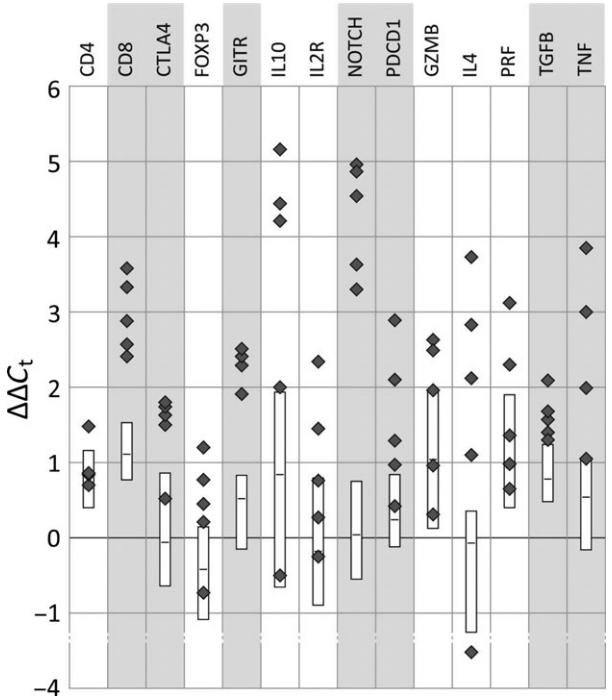

Figure 1 Gene expression in hand and kidney transplant recipients. Data are presented as ΔΔCt (mean $\Delta Ct_{ref} - \Delta Ct_{sample}$, where $\Delta Ct = Ct_{18S} - Ct_{gene}$, Ct is the cycle threshold value and defines the calculated cycle number, in which the fluorescence measured during PCR increases over the preset threshold value). Diamond—data for each HTx recipient, box—25th–75th percentile of KTx group, dash—median of KTx group, gray background—significant difference between HTx and KTx.

Moreover, an immunomodulatory molecular pattern with upregulation of *IL10*, *GITR*, and *PDCD1* was noted in HTx when compared to KTx or controls.

Vascularized composite allotransplantation contains skin, which is one of the most immunogenic tissue types, with more T effector cells than in blood [10,11]. On the other hand, VCA, unlike solid allografts, may be regarded as a vascularized bone marrow transplant with donor hematopoietic stem cells engrafting in the recipient bone marrow and thus promoting tolerance to donor-specific tissues [12–15]. However, the spontaneous development of mixed chimerism in VCA recipients is not frequent [16], and donor-specific hyporesponsiveness did not develop clinically in VCA [12,17].

Recipients of VCA are treated with immunosuppression reflecting that used in organ transplantation; however, it proves ineffective in preventing early rejection episodes in 100% of hand transplant recipients [18,19]. Because of high rate of rejection episodes, the total immunosuppression burden in VCA is generally high so it may be expected that the state of partial immune quiescence could develop in stable VCA recipients.

Despite the possibility of HTx recipients becoming quiescent, we observed that the following potent markers of the immune activation [20–22] were upregulated in HTx compared to controls and KTx: *NOTCH*, *TNF*, and *CD8*.

Notch is a signaling protein associated with differentiation of T and B cells and organ allograft rejection [22–24] and also in chronic rejection of animal VCA [25]. There are no reports of peripheral blood Notch signaling in hand transplant recipients except for our present report. TNF-alpha in kidney transplantation [26] as well as in animal model of limb transplantation was one of the best predictors of rejection and also preceded histopathological alterations [27]. CD8 identifies cytotoxic/suppressor T cells. In VCA rat model, the cellular infiltrate predominantly comprised mostly $CD8^+$ T cells but only the $CD4^+/CD8^+$ ratio increased with severity of rejection in skin biopsies [28]; however, in clinical setting, a pronounced $CD8^+$ infiltrate in more severe rejection was noted [29]. Long-lasting antigenic stimulation results in accumulation of late differentiated heterogeneous population of $CD3^+CD8^+CD28^-$ cells with suppressive or even more pronounced cytotoxic potential. Operationally tolerant or stable KTx presented lower levels of $CD3^+CD8^+CD28^-$ than recipients with chronic rejection [30,31]. In our study, both the HTx and KTx groups presented an expanded population of $CD3^+CD8^+CD28^-$ compared to controls.

Although in the first part of our research, we demonstrated increased proinflammatory, rejection-related status of the stable hand transplant recipients at the cellular level, we also noted that it is counterbalanced by the cell populations and expression of genes related to T regulatory cells phenotype.

Several subpopulations of lymphocytes have been shown to be involved in immune regulation, especially regulatory T cells ($CD4^+CD25^{high}CD127^{low}FOXP3^+$; Tregs) [5,30,32–34]. In stable KTx, an increased number of Tregs in the peripheral blood compared with recipients with chronic graft dysfunction was reported [35] but also Tregs were observed during acute rejection of transplanted organs [36]. Data concerning the prognostic value of Treg presence in VCA are inconsistent. On animal VCA model, blood Treg values did not differ rejection from rejection-free groups [37]. However, human Tregs were demonstrated to prevent the rejection of a skin allograft *in vivo* in an animal model of VCA [38].

To show functional immunomodulatory path of immune response, we also examined phenotypic gene markers of Tregs: *FOXP3*, *CTLA4*, and *GITR* with genes coding mediators of Treg functions: *TGFB*, *IL10*, and *PDCD1*.

In KTx, increased blood expression of FOXP3 was seen among tolerant recipients compared to rejection group [39,40]. TGF-beta can facilitate conversion of nonregulatory T cells to a suppressive phenotype [41] prolonging allograft survival in a skin transplant model [42]. Increased expression of TGF-beta in a biopsy of a well-accepted human composite tissue allograft was observed [43]. Nevertheless, the recent study on operationally tolerant KTx revealed that increased blood *TGFB1* expression was a marker of tolerance, whereas the expression of *FOXP3* and *IL10* did not show any significant difference between healthy controls and stable graft recipients [44]. In stable, tolerant VCA recipients, skin expression of $FOXP3^+$ was observed [43,45] but also in biopsy specimens showing rejection grades I–III [28].

In our study, despite high immunosuppression load, HTx present the same Treg level as healthy controls, which is not the case in KTx that presented lower Treg cell counts. Hand transplant recipients did not differ according to *FOXP3* expression levels from stable kidney transplant recipients or healthy controls; however, other functional markers of Tregs (*IL10*, *GITR*, and *PDCD1*) were upregulated in HTx compared to KTx or controls. GITR, highly expressed on Tregs, serves as a counterbalance to CTLA-4 engagement [46] with high expression levels in operationally tolerant kidney transplant recipients [6]. Inhibition of the CTLA-4 pathway was demonstrated to be effective in achieving immunosuppression in composite tissue transplants [47]. Together with PDCD1 and CTLA-4, IL-10 belongs to functional markers of Tregs and inhibits T-cell responses [32,48].

According to our knowledge, this is the first published report concerning increased expression of *IL10*, *GITR*, and *PDCD1* expression in stable HTx, suggesting robust immunoregulatory potential.

In summary, we have demonstrated for the first time that stable hand transplant recipients with no signs of rejection do not present "immune quiescence." As an effect of persistent immune activation, an immunomodulation-associated molecular pattern is observed probably as a safety mechanism to prevent uncontrolled overactivation of the immune response. Moreover, the mechanisms are not silenced by a high immunosuppression load in hand transplant recipients and are enhanced during repeated rejection episodes. The hypothesis is supported by growing data on chronic rejection cases among composite tissue allograft recipients [2].

The limitation of our study includes the small number of HTx that reflects the worldwide scarcity of VCA procedures. There was no possibility to match the HTx and KTx according to total immunosuppression load. Many skin rejection episodes resulted in multiple increases in steroid dosage, whereas repeating episodes of kidney rejection usually lead to allograft loss not to long-term stable graft function.

Conclusion

Despite the small size of the study group, we have shown that stable hand transplant recipients exhibit persistent immune activation with rejection-related gene expression pattern counterbalanced by secondary induction of immunomodulatory mechanisms.

Authorship

DK: participated in research design, in the writing of the manuscript, in the performance of the research, and in data analysis. KK-K: participated in research design, in the writing of the manuscript, in the performance of the research, and in data analysis. MK, AC, JJ, MŻ, MM, MB, MB, AG and PD: participated in the performance of the research. MK: participated in research design. MK: participated in research design, in the writing of the manuscript, and in data analysis.

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Conflicts of interest

The authors declare no conflicts of interest.

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