Host-derived Endothelial Cells replacing Graft Endothelium in Transplant Arteriosclerosis are Primarily Non-Bone Marrow derived

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Abstract

Neointimal lesions in chronic transplant dysfunction consist of vascular smooth muscle (VSM) cells intermingled with inflammatory cells, and are covered with endothelial cells (EC’s). The graft EC’s of a transplanted organ are thought to be the major target for graft rejection. The origin of EC’s after transplantation has been studied for several decades, and both donor-origin and host-origin have been described. EC replacement appears to be correlated to vascular injury. We recently showed complete EC replacement by host-derived endothelium in aortic allografts transplanted in rats. The source of those host-derived EC’s contributing to re-endothelialization of graft’s vessels is still a matter of debate. Here we used aortic allografts transplanted in MHC-mismatched bone marrow chimeric rats to determine the contribution of bone marrow (BM)-derived and non-BM-derived host-EC’s in the development of TA. Confocal laser scanning microscopy demonstrated that the neointimal EC’s (and VSM cells) are predominantly non-BM-derived.
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Introduction

Long-term survival of solid-organ transplants is limited as a result of chronic transplant dysfunction (CTD) which is histologically characterized by progressive concentric intimal thickening (transplant arteriosclerosis, TA). Intimal lesions consist of vascular smooth muscle (VSM) cells intermingled with inflammatory cells\(^1,2\). Persistent alloreactivity against the graft seems to be the predominant factor in the development of TA, however, precise mechanisms involved in the pathogenesis of TA still remain obscure. Current thinking on the process of TA holds that in response to signals from inflammatory cells and activated/damaged graft endothelium (cytokines, growth factors), medial VSM cells migrate into the subendothelial space beneath the graft endothelium\(^3,4\). Neointimal endothelial cells (EC’s) and VSM cells are therefore generally presumed to originate from the donor. In 1959, Woodruff\(^5\), and later expanded by Medawar\(^6\), proposed that replacement of graft endothelium with host-derived EC’s results in graft adaptation, thereby reducing the immunogenicity of the graft. According to this hypothesis, graft adaptation should result in improved long-term graft survival. Since then, the origin of EC’s after transplantation has been studied extensively, and both data supporting donor-origin\(^7-10\) and host-origin\(^11-15\) have been described. The proposed beneficial effect of EC replacement by host-derived cells, however, was not demonstrated in these studies. In contrast, Lagaaij et al. recently showed that donor EC replacement with host-derived endothelium was correlated with the severity of vascular rejection in human kidney allografts, suggesting that EC replacement occurs in response to vascular damage\(^16\). Whether EC replacement influences the development of TA is unclear, however, a worse prognosis instead of graft adaptation is not unreasonable.

We showed complete EC replacement by host-derived endothelium in aortic allografts transplanted in non-immunosuppressed rats, whereas in cardiac allografts transplanted in immune modulated rats graft endothelium was not replaced by host-derived EC’s\(^17,18\). Data on the origin of EC’s reported so far thus indicate that EC replacement might occur after allogeneic organ transplantation and that replacement seems to be a repair-mechanism upon vascular injury. Whether or not donor endothelium will be replaced by host-derived EC’s seems to depend on the extent of the vascular injury.

The anatomical source of the host-derived EC’s which replace donor endothelium in the graft upon injury is still unknown. Circulating EC progenitor cells (EPC’s) recruited from the bone marrow (BM) would be suitable candidates, since recirculating BM derived CD34\(^+\) endothelial cell progenitors have been identified in the peripheral blood of human individuals\(^19\). Moreover, it was recently demonstrated that Flk-1 positive cells derived from embryonic stem cells can differentiate into both EC’s and VSM cells\(^20\). However, also cells which are not recruited from the BM, such as circulating endothelial cells (CEC’s), may give rise to the neointimal EC’s in TA\(^21\).

Here we used MHC-mismatched aortic allografts transplanted in allogeneic bone marrow chimeric rats to determine
whether neointimal EC’s are BM or non-BM-derived. Using MHC-class I haplotype specific staining and confocal laser scanning microscopy we demonstrate that the neointimal EC’s in advanced intimal lesions are predominantly non-BM derived. However, in developing lesions, in which the donor EC’s had partly disappeared, we observed among the scattered distributed host-derived EC’s some EC’s which originated from the BM-compartment. These data indicate that BM-derived EC’s may contribute to the re-endothelialization process during the early stages of TA development. However, the overall contribution of those cells to EC replacement in intimal lesions is probably minimal since BM-derived EC’s can not be detected in advanced TA.

Methods

Rats
Specific-pathogen free male Lew (RT-1\(^l\)) and BN (RT-1\(^n\)) were used as BM donors or recipients and as aorta recipients. Male DA (RT-1\(^{avl}\)) rats were used as aortic allograft donors. All rats were aged 7-10 wk and were purchased from Harlan (Zeist, The Netherlands). Animals were maintained under clean conventional conditions and fed standard rat chow and acidified water ad libitum. All animals received humane care in compliance with the “Principles of Laboratory Animal Care” (NIH Publication No.86-23, revised 1985) and the Dutch Law on Experimental Animal Care.

Bone marrow transplantation
Both femora and tibiae of the donor rats were excised and surrounding muscle and connective tissue were removed. Both ends were removed with small pincers, and the BM was flushed with RPMI 1640 (Life Technologies) using a syringe with a needle. Clusters were disaggregated by vigorous pipetting and the cell suspension was filtered using nylon gauze. BM cells were washed, counted and set to a concentration of 2x10^8 cells/ml. Male recipient rats aged 7-10 wk were lethally \(\gamma\)-irradiated (BN 8.5 Gy and Lew 9.5 Gy) using a \(^{137}\)Cesium source (IBL 673, CIS Bio International). Irradiated BN and Lew hosts received 1x10^8 Lew and BN BM cells respectively by tail vein injection. Four to six weeks after bone marrow transplantation, chimerism was determined in the peripheral blood by flowcytometry. If complete chimerism was observed aortic transplantation of DA allografts into the chimeric hosts was performed.

Aortic transplantation
Aortic allografts (10-12 mm) were transplanted orthotopically as described previously\(^{22}\). Total ischemic time was consistently less than 30 min. Transplantations were performed in the DA to Lew\(_{BM}\)->BN and DA to BN\(_{BM}\)->Lew strain combinations.

Flowcytometry
To determine the level of chimerism at the time of aortic transplantation (4-6 weeks after BM transplantation), 0.5 ml of heparinized blood was obtained from the BM transplant recipients by cardiac puncture. The blood samples were 1:1 diluted with PBS and subsequently centrifuged (2500 rpm, 20 min). Buffy coat mononuclear cells (MNC’s) were isolated and remaining erythrocytes were
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lysed in NH₄Cl lysis-buffer. After centrifugation (1200 rpm, 10 min) the cells were resuspended in PBS and counted. At the time of sacrifice three months after aortic transplantation, aortic allografts were explanted and processed for histology. Moreover, to determine the level of chimerism in the BM compartment, BM cells were isolated as described above. MNC’s and BM cells (0.5x10⁶/sample) were centrifuged in a 96-well microtiter plate (1050 rpm, 3 min at 4°C) and resuspended in PBS containing 2% newborn calf serum, 0.01% NaN₃, and a mixture of monoclonal antibodies (mAb’s). The following mAb’s were used: OX18 (pan MHC class I)²³, OX27 (BN [RT-1ª] MHC class I haplotype)²⁴, and OX7 (CD90, Thy-1)²⁵.

Cells were analyzed on an Elite flow-cytometer (Coulter), and data analysis was conducted with FlowJo (for Macintosh, release 3.4 software package). Within the forward and side scatter diagram the lymphocyte population was gated and within this population, the expression of Thy-1 (among others expressed on hematopoietic stem cells, early myeloid and erythroid cells, and immature B cells), OX18 and OX27 was analyzed. Positive OX18 staining confirmed MHC class I expression, whereas positive OX27 staining indicated BN origin of these cells.

Confocal laser scanning microscopy

Three months after transplantation, the aortic grafts were explanted and immediately frozen in liquid nitrogen and stored at -80°C for cryostat sections. To analyze the development of TA, an indirect immunofluorescence double staining was performed using mAb’s asm-1 (IgG₂a, VSM cell α-actin)²⁶,²⁷ and RECA-1 (IgG₁, endothelium)²⁸. The origin of neointimal EC’s in grafts transplanted in the BM chimeric rats was analyzed using the following mAb’s: OX18 (IgG₁, pan MHC class I)²³, OX27 (IgG₂a, BN [RT-1ª] class I)²⁴, and MN4-91-6 (IgG₁, DA [RT-1ª] MHC class I haplotype)²⁹. The following combinations of antibodies were used for double-staining: asm-1 & OX18, asm-1 & MN4-91-6, and OX27 & RECA-1. Second-step antibodies consisted of FITC conjugated goat-anti-mouse IgG₂a and TRITC conjugated goat-anti-mouse IgG₁ antibodies (Southern Biotechnologies). Sections were DAPI (Molecular Probes) counter-stained, embedded in Vectashield (Vector Laboratories), and subsequently analyzed with a confocal laser scanning microscope (Leica).

Results

Chimerism after bone marrow transplantation

Four to six weeks after BM transplantation, we analyzed the level of chimerism in the peripheral blood by MHC class I haplotype specific flowcytometry. In all BN hosts transplanted with Lew derived BM cells (LewBM->BN) (n=4), <1% of the OX18⁺ leukocytes was OX27⁺ which was identical to Lew control rats, thus indicating that the peripheral blood was completely repopulated with BM-donor (Lew, OX27⁻) derived cells. Moreover, the total number of lymphocytes and subset distribution was normal compared to age-matched BN controls (not shown). In the Lew hosts transplanted with BN BM cells (BNBM->Lew) (n=4) peripheral blood
cellularity had also restored to normal levels at six weeks after irradiation and BM transplantation. However, only one animal displayed complete chimerism, e.g. all OX18+ cells showing the OX27+ BN-phenotype, whereas the other rats displayed split-chimerism. These latter animals were not used for further analysis. Stable bone marrow chimerism was confirmed by performing the same flowcytometric analysis on BM cells isolated from the chimeric aortic allograft recipients at the time of sacrifice, three months after aortic transplantation. In normal BN BM, all cells were OX27+ (Fig. 1A), whereas in normal Lew BM, all cells were OX27- (Fig. 1B). Although OX27, Lew BM cells clearly expressed MHC class I antigens as indicated by positive OX18 staining (not shown). Also in the BM chimeric rats, MHC class I expression was present on BM cells as indicated by positive OX18 staining (not shown). However, Lew<sub>BM</sub>->BN rats contained no BN-derived (OX27+) cells in the BM compartment, indicating that stable chimerism was established (Fig. 1C). In the BN<sub>BM</sub>->Lew rat, all cells in the BM compartment were BN-derived (OX27+) indicating that also in this combination stable chimerism was established (Fig. 1D).

**Origin of neointimal EC’s and VSM cells**

At 3 months after DA aortic allografting the Lew<sub>BM</sub>->BN rats had developed

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**Figure 1.** Allogeneic bone marrow (BM) transplantation resulted in the establishment of complete stable chimerism. The level of chimerism was determined three months after aortic transplantation by FACS analysis of BM cells. BM transplantation was performed in the Lew to BN (Lew<sub>BM</sub>->BN) and BN to Lew (BN<sub>BM</sub>->Lew) strain combinations. Fluochrome labeled mAb’s OX7 (PE) and OX27 (FITC) were used to detect lymphoid and myeloid cells, and BN MHC class I, respectively. Normal BN (A) and Lew (B) BM cells were used as controls. OX27- cells were only detected in BN BM cells, whereas Lew BM cells were OX27+. BM cells in Lew<sub>BM</sub>->BN rats (C) were OX27-, indicating that no residual BN-derived cells were present and complete stable chimerism had been established. BM cells in BN<sub>BM</sub>->Lew rat (D) were OX27+, indicating that also in this combination complete stable chimerism had been established.
marked TA consisting of a thick layer of \(\alpha\)-actin positive VSM cells, which was covered with endothelium at the luminal surface (Fig. 2A). The amount of neointima formation in the chimeric rats did not differ significantly from that observed in DA allografts transplanted in normal, non-chimeric, BN hosts (not shown). The neointimal EC’s (and VSM cells) were not of donor origin since they did not stain with the antibody MN4-91-6, which is specific for DA (donor-type) MHC class I (Fig. 2B). Surprisingly, some residual medial VSM cells showed weak but positive staining indicating graft origin of these cells. Medial VSM cells do normally not express MHC class I antigens (not shown). The negative staining of neointimal EC’s in Fig. 2B was not due to a lack of MHC class I expression on these cells, since staining with the pan MHC class I antibody OX18 gave a clear signal on EC’s, as well as on residual medial VSM cells, thus confirming the observation with mAb MN4-91-6 (Fig. 2C and 2D). Again to our surprise also some neointimal VSM cells displayed low but positive MHC class I expression. Neointimal VSM cells (Fig. 2E) and EC’s (Fig. 2F) were of host-origin as evidenced by positive OX27 (host-type) staining. Staining for \(\alpha\)-actin and MHC class I on serial tissue sections revealed co-localization of these markers thus ensuring that the OX27+ neointimal cells were indeed VSM cells (not shown). Since the bone marrow of these Lew\textsubscript{BM} \(\rightarrow\) BN rats was completely replaced with OX27- cells (see above), such OX27+ neointimal EC’s and VSM cells must be non-marrow derived.

Although the majority of the neointimal EC’s (and VSM cells) appeared to originate from the host’s non-marrow compartment, we could not exclude that also some BM-derived EC’s were part of the EC layer covering the neointimal lesion. Therefore, we performed the same type of analysis on a DA aortic allograft transplanted in a BM-chimeric Lew host reconstituted with BN BM-cells (BN\textsubscript{BM} \(\rightarrow\) Lew). Although at 3 months after transplantation the gross morphology of the aortic allograft appeared to be relatively normal, large parts of the internal elastic lamina were devoid of EC’s, while other parts were covered with stretches of EC’s (Fig. 3A). Moreover, also clusters of EC’s adhering to the internal elastic lamina were present (Fig. 3B) which are absent in normal, non-transplanted, aortic tissue. Staining for donor-type MHC class I using antibody MN4-91-6 revealed that positive cells (graft-origin) were still present, indicating that not all donor EC’s had been destroyed yet in this aortic allograft (Fig. 3C). Moreover, intermingled with the remaining graft-derived EC’s, also MN4-91-6 negative cells, suggesting host-origin, were present (Fig. 3C). The EC’s localized in clusters tended to be MN4-91-6 negative, suggesting host-origin of these cells (not shown). RECA-1 staining on serial sections confirmed that both cells shown in Fig. 3C are EC’s (Fig. 3D). The negative MN4-91-6 staining of one of the EC’s in Fig. 3C was not due to a lack of MHC class I expression, since staining with the pan MHC class I antibody OX18 gave a clear signal on all EC’s (Fig. 3E). These results further indicate host-origin of the MN4-91-6 negative EC’s. RECA-1 and OX27 (BM origin) double staining revealed that among the host-derived EC’s some double positive cells were present,
Figure 2. Confocal laser scanning microscopy indicated host-origin of neointimal EC’s and VSM cells in DA aortic allografts transplanted in Lew BM->BN rats and analyzed 3 months after transplantation. (A) Staining for $\alpha$-actin [asm-1, green] and EC’s [RECA-1, red]. Arrowheads indicate RECA-1 positive EC’s and the asterisk indicates $\alpha$-actin positive neointimal VSM cells (magn. x2520). (B) Staining for $\alpha$-actin [asm-1, green] and aorta donor (DA) MHC class I [MN4-91-6, red]. Asterisk indicates remaining medial VSM cells which are donor MHC class I positive. Double asterisks indicate $\alpha$-actin positive, MN4-91-6 negative, neointimal VSM cells, indicating host-origin (magn. x1260). (C) Staining for $\alpha$-actin [asm-1, green] and MHC class I [OX18, red]. Asterisk indicate MHC class I positive, $\alpha$-actin negative, remaining medial VSM cells. Double asterisks indicate MHC class I positive, $\alpha$-actin positive, neointimal VSM cells (magn. x1260). (D) High power magnification of (C). Arrowheads indicate MHC class I positive EC’s and the asterisk indicates MHC class I expression by $\alpha$-actin positive neointimal VSM cells (magn. x2520). (E) Staining for host (BN) MHC class I [OX27, green] and EC’s [RECA-1, red]. Asterisk indicates OX27 positive neointimal VSM cells, indicating host, non-BM, origin (magn. x630). (F) High power magnification of (E). Arrowheads indicate RECA-1 positive, OX27 positive, EC’s, indicating host, non-BM, origin (magn. x2520). Abbreviations: a, adventitia; m, media; ni, neointima.
Figure 3. Confocal laser scanning microscopy of a DA aortic allograft transplanted in the BN subrenal mesonephric bone-marrow chimeric rat and analyzed 3 months after transplantation revealed bone-marrow origin of EC’s. (A) Staining for α-actin [asm-1, green] and EC’s [RECA-1, red]. Asterisk indicates α-actin positive medial VSM cells. Arrowheads indicate stretches of RECA-1 positive EC’s, whereas the arrow indicates a cluster of RECA-1 positive EC’s attached to the internal elastic lamina. Note that other parts of the internal elastic lamina are devoid of EC’s (magn. x1260). (B) High power magnification of (A). Arrow indicates a cluster of RECA-1 positive EC’s attached to the internal elastic lamina. Asterisk indicates α-actin positive medial VSM cells (magn. x2520). (C) Staining for α-actin [asm-1, green] and aorta donor (DA) MHC class I [MN4-91-6, red]. Arrowhead indicates a donor MHC class I positive EC, indicating graft-origin. Arrow indicates a MN4-91-6 negative cell, suggesting host-origin. Asterisk indicates the nucleus of an α-actin positive medial VSM cell (magn. x5670). (D) Staining for α-actin [asm-1, green] and EC’s [RECA-1, red] on a serial section of (C). Arrowheads indicate RECA-1 positive staining, thereby confirming that the cells depicted in (C) are EC’s (magn. x5670). (E) Staining for α-actin [asm-1, green] and MHC class I [OX18, red]. Arrowheads indicate MHC class I positive EC’s. Asterisk indicates α-actin positive medial VSM cells (magn. x2520). (F) Staining for BM-donor (BN) MHC class I [OX27, green] and EC’s [RECA-1, red]. Arrowheads indicate RECA-1 positive EC’s, which are also OX27 positive (arrows), indicating that these EC’s are BM-derived (magn. x5040). Abbreviations: a, adventitia; iel, internal elastic lamina; m, media.
representing BM-derived endothelial cells (Fig. 3F).

**Discussion**

Several decades ago, EC replacement of graft endothelium was proposed to result in graft adaptation leading to improved long-term clinical outcome of solid organ transplants\(^5,6\). Replacement of graft endothelium by host-derived EC’s indeed has been reported by several groups, however, instead of improving long-term clinical outcome, EC replacement seems to be related to the severity of rejection and vascular damage\(^11-16\). These observations are in line with our own data in experimental transplant models in rats, in which we showed complete EC replacement of graft endothelium in aortic allografts transplanted in non-immunosuppressed rats whereas in immune modulated cardiac allograft recipients graft endothelium was preserved\(^18\).

To date, long-term survival of solid-organ transplants is limited as a result of chronic transplant dysfunction (CTD) which is histologically characterized by progressive concentric intimal thickening (transplant arteriosclerosis, TA)\(^1,2\). Although the role of EC replacement on the development of TA in clinical organ transplantation is unknown, our results clearly indicate that whether or not the graft is repopulated by host-derived EC’s, the development of TA proceeds\(^18\). This suggests that EC replacement is a response to vascular-damage, but is not necessary for the formation of a hyperplastic neointima per se.

The source of host-derived EC’s repopulating the graft’s blood vessels upon vascular injury is still a matter of debate and several possibilities have been proposed\(^30,31\). Circulating EC’s, derived from the vascular bed, could be recruited to sites in need of re-endothelialization, i.e. the damaged vascular wall. A similar role has been suggested for BM-derived endothelial precursor cells (EPC’s). Recirculating BM-derived EPC’s have been identified in the peripheral blood of humans\(^32,33\). Moreover, in animal models it has been shown that the frequency of such circulating EPC’s increases in response to regional ischemia, thereby contributing to neovascularization of ischemic regions\(^34,35\).

Here we analyzed the contribution of BM-derived and non-BM-derived EC’s in the re-endothelialization process and development of TA in aortic allografts transplanted in MHC-incompatible BM-chimeric recipient rats. Using MHC class I haplotype specific immunofluorescence and confocal laser scanning microscopy we showed that virtually all EC’s covering the neointimal lesions in advanced TA are host-derived, but do not originate from the BM. Most likely, the host-derived neointimal EC’s are recruited from a pool of CEC’s which have been shown to be present in human blood in a low frequency\(^21\). In contrast to advanced neointimal lesions, characterized by a thick layer of \(\alpha\)-actin positive and MHC class I negative VSM cells, some BM-derived EC’s were detected in an aortic allograft in which the neointima had not yet developed. In this particular allograft, the vascular media still contained \(\alpha\)-actin positive VSM cells which had not disappeared as a result of medial VSM cell necrosis. The graft endothelium covering the internal elastic lamina was injured and partly replaced by host-derived EC’s. Although not many,
some BM-derived EC’s were identified. Jackson et al. recently demonstrated regeneration of vascular endothelium by BM-derived adult stem cells in ische-
mically injured hearts in mice36. Although
BM-derived EC’s were detected in the is-
chmic regions, the prevalence of those
cells was about 3%.
In aortic allografts with advanced TA, the
vascular media had become acellular or
only contained some remaining graft-
derived VSM cells which had lost their
α-actin expression. Using a PCR-based
analysis we previously demonstrated
host-origin of neointimal VSM cells in TA
in aortic and cardiac allografts17,18 and
these results were now confirmed in the
aortic allografts by confocal laser
scanning microscopy. Moreover, similar
to the origin of host-derived EC’s, also
the neointimal VSM cells were primarily
non-BM-derived. Two recent papers by
Shimizu et al.37 and Li et al.38 analyzed
the contribution of BM-derived VSM
cells in the development of TA in mouse
aortic allografts. Using BM-chimeric
mice reconstituted with β-galactosidase-
expressing BM-cells, Shimizu et al. de-
monstrated that up to 10% of the
neointimal VSM cells were BM-derived.
In contrast, Li et al. were not able to detect
BM-derived neointimal VSM cells using
Y-chromosome specific in situ hybridi-
zation. Although also in the aortic trans-
plant model in mice graft endothelium is
completely replaced by host-derived EC’s
at 30 days after transplantation (S.M.
Ensminger, personal communication), the
issue of the origin of neointimal EC’s was
not addressed in these studies37,38,
probably due to technical reasons.
So, results from the literature and data
presented in this paper thus indicate that
neointimal VSM cells and EC’s are
primarily derived from the non-BM-
compartment. Migration of host-derived
EC’s and VSM cells from the recipient
side of anastomosis might be a possible
source of these cells as suggested by Aziz
et al.39. Alternatively, CEC’s detached
from host blood vessels can provide a
source of EC’s contributing to the re-
endothelialization process of allografts.
Circulating VSM progenitor cells have
not been identified so far. However
Bucala et al. reported the existence of a
non-BM-derived circulating cell popu-
lation that specifically enters sites of tissue
injury40. Moreover, Kouchi et al. de-
scribed the existence of bloodstream
originated α-actin positive VSM cells41.
In the developing neointima in aortic
allografts, a scattered distribution of
α-actin positive cells adhering to the de-
veloping neointima was observed,
suggesting bloodstream origin of these
cells42. Moreover, the scattered adherence
of host-derived EC’s to the internal elastic
lamina in the developing lesion as
mentioned in this paper also suggests
bloodstream origin of the EC’s. We
therefore propose bloodstream origin of
neointimal EC’s and VSM cells derived
from a non-BM source in the host.
In conclusion, although the BM can
provide EC and VSM cell progenitor
cells, the contribution of those cells in the
development of TA and probably also
other vascular repair processes, seems to
be minimal. Therefore, the biological
importance and the functional relevance
of BM-derived EC and VSM cell
progenitor cells in vascular wall repair
processes is questionable. Non-BM-
derived host EC’s and VSM cells seem to
be more important in these processes. One
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should focus therefore on these cells when trying to unravel the pathogenesis of TA and to develop new therapeutic inter-
ventions to prevent or treat TA after solid organ transplantation.

References


29. Pockley AG, Reid SD, and Bowles MJ. An enzyme immunoassay for rat soluble MHC class I molecules (RT1a) and the release of soluble class I from mitogenically stimulated mononuclear cells. *Immunol. Invest.* 1995; 24:679-687.


