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Mesenchymal stem cells protect endothelial cells from cytotoxic T lymphocyte induced lysis

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Abstract:

The integrity of the vasculature plays an important role in the success of allogeneic organ and haematopoietic stem cell transplantation. Endothelial cells (EC) have previously been shown to be the target of activated cytotoxic T lymphocytes (CTL) resulting in extensive cell lysis. Mesenchymal stromal or stem cells (MSC) are multipotent cells which can be isolated from multiple sites, each demonstrating immunomodulatory capabilities. They are explored herein for their potential to protect EC from CTL targeted lysis.

$CD8^+$ T cells isolated from human PBMC were stimulated with mitotically inactive cells of a human microvascular endothelial cell line (CDC/EU.HMEC-1, further referred to as HMEC) for 7 days. Target HMEC were cultured in the presence or absence of MSC for 24 hours before exposure to activated allogeneic CTL for 4 hours. EC were then analysed for cytotoxic lysis by flow cytometry.

Culture of HMEC with MSC in the efferent immune phase (24 h before the assay) led to a decrease in HMEC lysis. This protection was lost when MSC were cultured in a transwell system, where contact between the MSC was withdrawn. Further analysis suggested that prostaglandin E2 (PGE2) has a role to play in MSC abrogation of lysis. The efficacy of multiple sources of MSC was also confirmed and the collaborative effect of MSC and the endothelium protective drug defibrotide was determined, with defibrotide enhancing the protection provided by MSC. These results support the use of MSC as an adjuvant cellular therapeutic in transplant medicine, be it alone or in conjunction with EC protective agents such as Defibrotide.

39 **Abbreviations**

40 EC: Endothelial cell

41 HMEC: CDC/EU.HMEC-1, an immortalized human microvascular endothelial cell line

42 IL-2: Interleukin 2

43 PBMC: Peripheral blood mononuclear cells

44 CTL: Cytotoxic T lymphocyte

45 BM-MSC: Bone marrow derived mesenchymal stem cell

46 PVSC: Perivascular (umbilical artery derived) MSC

47 UC-MSC: Umbilical cord matrix derived MSC

48 PGE2: Prostaglandin E2

49

50 **Highlights**

51 - MSC protect EC from CTL lysis
52 - Cell-to-cell contact and the release of PGE2 are involved in MSC protection
53 - MSC from different sources achieve similar results
54 - Defibrotide adds to the protection observed in MSC co-culture

55

56 **Introduction**

57 Allogeneic transplantation of organs or haematopoietic stem cells is often the only
58 therapeutic option for end-stage organ disease or leukaemia, respectively. However, due to
59 disparities in major and minor histocompatibility antigens between donor and recipient, severe
60 inflammatory complications can occur, requiring immunosuppression. Unfortunately,
61 immunosuppression seriously affects the quality of life of patients and is a major threat to the
62 health economy. The vascular endothelium is the primary contact between foreign and self, and
63 damage to vascular endothelial cells represents the primary adverse event finally resulting in loss
64 of organ function. This central role and early appearance of endothelial damage makes it an ideal
65 target for early therapeutic intervention. Endothelial damage to the graft from ischemic
66 reperfusion injury in the case of solid organ transplantation (Boyle et al., 1997) or to the host
67 from conditioning in allogeneic haematopoietic stem cell transplantation (Eissner et al., 1995,
68 Paris et al., 2001), ultimately leads to loss of organ function and transplant failure. Damage to the
69 mucosal barriers results in the expression of damage associated molecular patterns and the
70 translocation of foreign antigen such as LPS across damaged membranes. This combined with
71 the exposure to inflammatory mediators results in the development of an immunologically active
72 state, rendering EC to become antigen-resenting cells. Previous work published by our group
73 demonstrated the destructive repercussions of the invasion of cytotoxic T lymphocytes (CTL)
74 which specifically target endothelial cells (EC) (Eissner et al., 2011). Importantly, these CTL
75 show an unusual phenotype (CD28-negative) and are peculiarly enhanced in their cytotoxic
76 activity by the co-culture with FoxP3^{+} regulatory T cells (Treg cells) (Eissner et al., 2011),
77 suggesting that the currently available immunomodulatory mechanisms cannot protect EC
78 against these effector cells.

79 Over the last 10 years the clinical use of bone marrow derived mesenchymal stem cells
80 (BM-MSC) as a cellular therapeutic has risen dramatically, with over 350 clinical trials
81 registered across a broad spectrum of diseases (Wei et al., 2013). Early work looked at MSC for
82 their capacity to differentiate into multiple lineages (Friedenstein et al., 1966, Pittenger et al.,
83 1999), testing their ability to replace damaged tissue by *in situ* differentiation. It is now,
84 however, more commonly accepted that MSC home to the site of injury and influence the local
85 environment through the release of soluble factors (Caplan and Dennis, 2006, Chen et al., 2008).
86 The same can be observed in vascular biology where soluble mediators, such as VEGF and IGF,
87 released by MSC in a model of myocardial infarction promote tube formation and protect cardiac
88 cells against apoptosis (Sadat et al., 2007). Bader *et al*. also showed MSC can protect EC from
89 apoptosis in hypoxic conditions associated with ischemic disease, improving viability and
90 proliferation (Bader et al., 2014). These results support the use of MSC in transplant medicine,
91 targeting protection or repair of the endothelium. Use of MSC in combination therapy has also
92 become common practice, with publications into renal fibrosis (Huuskes et al., 2015) and clinical
93 trials in GvHD (Le Blanc et al., 2008), siting increased attenuation of pathology.

94 Defibrotide is a polydisperse mixture of mostly single stranded oligonucleotides with
95 anti-inflammatory, anti-ischemic, pro-fibrinolytic, and antithrombotic functions (Guglielmelli et
96 al., 2012). As of 2013 it has been approved by the European Medicines Agency as a treatment
97 for hepatic veno-occlusive disease (VOD), a severe endothelial complication following
98 allogeneic haematopoietic stem cell transplantation. Treatment of EC with the chemotherapeutic
99 drug fludarabine, also used in pre-transplant conditioning, results in apoptosis and allogeneic
100 activation. The addition of defibrotide rescued EC from these adverse events without interfering
101 with the desirable anti-leukemic and anti-T cell proliferative effects of the drug (Eissner et al.,

102 2002). These observations prompted investigation into not only the efficacy of MSC, but the
103 examination of defibrotide and MSC as a combination therapy in a cytotoxicity assay.

104

105 **Materials and methods**

106 *Cell culture and reagents*

107 The human dermal microvascular endothelial cell line CDC/EU.HMEC-1 (further referred to as
108 HMEC) was kindly provided by the Centers for Disease Control and Prevention (Atlanta,
109 Georgia, USA) (Ades et al., 1992). HMEC were cultured in MCDB131 medium, supplemented
110 with 15% fetal calf serum (FCS), 1 µg/mL hydrocortisone (Sigma, Deisenhofen, Germany), 10
111 ng/mL epidermal growth factor (Collaborative Biochemical Products, Bedford, MA, USA) and
112 antibiotics. Defibrotide was kindly provided by Dr. Terenzio Ignoni (Gentium, SpA., a Jazz
113 Pharmaceuticals Company, Villa Guardia (CO), Italy). Bone marrow derived MSC (BM-MSC)
114 were provided by Apceth GmbH & Co.KG, by the Department of Internal Medicine III,
115 Regensburg University Medical Centre, Regensburg, Germany, or purchased from Pelobiotech
116 (PELOBiotech GmbH, Planegg, Germany), Perivascular mesenchymal stem cells (PVSC) were
117 isolated from the perivascular region of umbilical cord arteries (Department of Gynaecology,
118 Wolfart-Klinik (Gräfelfing, Germany)) and Amniotic membrane-derived MSC (hAMSC) were
119 kindly provided by Ornella Parolini, Centro di Ricerca E. Menni, Fomdazione Poliambulanza,
120 Brescia, Italy. All MSC were cultured in Human Xeno-free Mesenchymal Stem Cell Medium
121 (PELOBiotech GmbH, Planegg, Germany) containing supplement and 1%
122 Penicillin/streptomycin. T lymphocytes were isolated form PBMC from either leukapharesis
123 products by the Department of Clinical Chemistry and Transfusion Medicine (Regensburg
124 University Medical Centre, Regensburg, Germany) or from buffy coats by the Irish Blood
125 Transfusion Service and were cultured in RPMI containing 10% FCS, 1% L-glutamine, 1%
126 Penicillin/streptomycin and 300U/ml of Interleukin 2 (IL-2);(Novartis, Basel, Switzerland). All
127 reagents have been purchased from Life Technologies unless stated otherwise. All biological

128 samples were obtained with informed consent according to guidelines of the local Ethical review
129 committees.

130 *Separation and culture of CD8 T cells*

131 $CD8^+$ T cells were isolated using MagCellect $CD8^+$ T cell isolation kit according to
132 manufacturer's instructions (R&D Systems, Minneapolis, MN, USA). Briefly, PBMC were
133 negatively selected by incubation with biotin antibody cocktail followed by incubation with
134 ferro-streptavidin and finally magnetic separation. Purity of isolation was determined by flow
135 cytometric analysis.

136 *Cytotoxicity Assay*

137 $CD8^+$ T cells were stimulated in a ratio of 1:1 with mitotically inactivated (50 µM
138 Silibinin, Sigma-Aldrich, Deisenhofen, Germany) HMEC for 7 days in the presence of IL-2 (300
139 U/mL). Cytotoxicity was assessed according to a well established protocol (Piriou et al., 2000).
140 Target HMEC were labelled with 30 µM 3,3´-dioctadecyloxacarbocyanine perchlorate
141 ($DIOC18_{(3)}$) by incubating at 37°C for 15min. HMEC were then cultured in the presence or
142 absence of MSC or drugs (day-1) for 24 hours followed by incubation with $CD8^+$ T cells at
143 descending effector to target ratios (20:1, 10:1 and 5:1) for another 4 hours. The percentage of
144 specific lysis determined by courter staining the HMEC with propidium iodide (PI);(0.2 µg/mL,
145 Sigma-Aldrich, Deisenhofen, Germany) and determining lysis by flow cytometry analysis (BD,
146 Oxford, UK). Defibrotide was added to the culture system at day-1 at a concentration of 25µM
147 and Indomethacin (Sigma-Aldrich, Deisenhofen, Germany) was used at a concentration of
148 40µM.

149

150 *Transwell Culture*

151 Transwell inserts for a 24 well plate with a 0.4μm pore size (Corning, Hazebrouck,
152 France) were used to separate the MSC from HMEC and CTL during the cytotoxicity assay.
153 $5x10^4$ $DIOC18_{(3)}$ labelled HMEC were seeded in the bottom of the 24 well plate, while $1x10^4$
154 BM-MSC were plated either in contact with the HMEC or in the upper chamber of the transwell
155 insert. The cells were cultured for 24 hours in HMEC medium. The medium was removed and
156 re-placed with CTL medium before CTL were added to the bottom well of the plates and
157 cultured for a further 4 hours. The HMEC were collected and counter stained with PI and
158 examined by flow cytometry.

159 *PGE ELISA*

160 The amount of PGE-2 produced during a cytotoxicity assay was measured using a
161 commercially available ELISA Kit from Cayman Chemical (Ann Arbour, MI, USA). The PGE-2
162 assay is based on the competition between PGE-2 and PGE-2 acetylcholinesterase (AChE)
163 conjugate (PGE-2 tracer) for a limited amount of PGE-2 monoclonal antibody. ELISA were
164 carried out according to manufacturer's instructions. The microplate (96 well polystyrene) was
165 pre-coated with goat anti-mouse polyclonal antibody. The standards and samples in triplicate and
166 controls in duplicate (50 μl) were added to the plate, and read at an absorbance of 405 nm using
167 a multiscan plate reader (LabSystems, Thermo-Fisher, Waltham, MA, USA).

168 *Statistical Analysis*

169 Statistical analysis was performed using GraphPad Prism™ software (GraphPad, San
170 Diego, CA, USA). The students paired t test was used when statistical analysis was required
171 between two experimental groups. One way ANOVA was used to test for statistical significance

172 of differences when multiple experimental groups were compared. Data are presented as the ±

173 standard error of the mean (SEM). P-values of $p<0.05$ (*), $p<0.01$ (**) or $p<0.001$ (***) were

174 considered statistically significant.

175 **Results**

176 **MSC protect endothelial cells against allogeneic CD8$^+$ CTL**

177 Previous data published by our group demonstrated the existence of a strictly EC-specific
178 CD8$^+$ T cell subpopulation that is peculiarly enhanced by the co-administration of Treg cells
179 (Eissner et al., 2011). Following up on this work, the present study looked to investigate the role
180 of MSC in protecting EC from targeted killing. Untouched CD8$^+$ T cells were isolated and co-
181 cultured with allogenic EC (HMEC) for 7 days. 24 hours before the cytotoxicity assay, HMEC
182 were stained with DIOC and cultured in the presence or absence of BM-MSC at a ratio of 5:1,
183 respectively. The cytotoxicity assay involves culturing the primed CTL with the treated or
184 untreated HMEC for 4h, staining with PI and analysing the expression of PI versus DIOC by
185 flow cytometry. When cultured in the presence of BM-MSC there was a significant decrease in
186 the extent of cell lysis elicited by the CTL. The CTL were titrated resulting in a titrated rate of
187 cell death, in each case the MSC reduced the extent of EC killing (Fig. 1A), with significance at
188 20:1 graphed as a bar chart (Fig. 1B), with the 10:1 and 5:1 ratios also reaching significance
189 (data not shown).

190 Following on from this work, efforts were made to determine the signalling mechanisms
191 involved in MSC protection of EC. However, before this could be achieved more needed to be
192 understood about the physical interactions between the cells in culture. The lysis of EC by CTL
193 was herein shown to be fully MHC Class I restricted, as pre-treatment of HMEC with w6/32, an
194 MAH Class I-restricted monoclonal antibody, prior to their encounter with CTL, completely
195 abrogated the CTL activity. The extent of lysis was reduced to that observed with MSC co-
196 culture (Supplementary Fig. 1). Lastly, to eliminate the possibility that MSC were simply

197 masking the contact sites on EC from the CTL by physically covering them, human heart explant
198 cells (HH7) as size-matched negative controls were used in the place of MSC in a cytotoxicity
199 assay. These heart explant cells failed to protect the EC from cell death thus negating the
200 possibility of MSC simply shrouding the EC (Supplementary Fig. 2).

201 **MSC protection requires contact**

202 MSC signalling has provoked a long running debate as to the importance of cell-cell
203 contact over the release of soluble factors. Results from transwell cytotoxicity experiments
204 performed herein showed that in the absence of cell contact MSC failed to protect HMEC from
205 CTL induced lysis (Fig 2A). The extent of HMEC lysis did not increase to levels observed in
206 untreated HMEC however, suggesting that MSC may release some soluble factor that infers
207 some modicum of protection but not to the extent of MSC in contact conditions.

208 The multitude of cytokines and stimulants released by MSC posed a problem in
209 narrowing down the potential candidate's eliciting the protective effects on EC. A cytokine array
210 on the supernatant from transwell experiments was preformed, also including the supernatant
211 from MSC in contact with $CD8^+$ T cells but in the absence of HMEC. There was little difference
212 between the supernatant from MSC in contact and those in transwell to suggest a candidate for
213 signalling. However, when PGE2 was examined by competitive ELISA there was a drop in the
214 level of PGE in the supernatant of cells from the transwell group (data not shown). Following on
215 from this work a COX inhibitor that prevents the synthesis of prostaglandins, indomethacin, was
216 added to the HMEC and MSC co-culture during the 24 hours prior to the cytotoxicity assay and
217 resulted in an increase in EC lysis despite the presence of MSC (Fig 2B).

218

219 **Protection evident in MSC from multiple sources**

220 BM-MSC are one of the most commonly used and studied groups of stem cells
221 predominantly due to the ease of isolation and their initial beneficial use in autologous MSC
222 therapy. However, MSC isolated from the umbilical cord and placenta have been shown to be
223 less immunogenic than BM-MSC while maintaining the same modulatory abilities (Pianta et al.,
224 2014). In order to determine whether MSC from different sources would work as well in this
225 system, MSC from the perivascular region of umbilical cord arteries (PVSC) and MSC of the
226 amniotic membrane (hAMSC) were tested in a cytotoxicity assay with various CTL donors.
227 Though BM-MSC provide the greatest protection on HMEC with only 24% of the cell
228 population undergoing lysis, PVSC (38%) and hAMSC (37%) still significantly reduce the level
229 of CTL lysis (Fig 3).

230 **Defibrotide enhances the protective effect of MSC**

231 Combination therapy, particularly in the case of cellular therapy has received a lot of
232 attention in the last number of years. Defibrotide as described above has been tested successful in
233 the treatment of VOD, its known interaction with endothelial cells led to a study of the drug in
234 the stabilisation of the endothelial compartment (Koehl et al., 2007). Previously published work
235 from our group defined a role for defibrotide in protecting the endothelium form CTL targeted
236 lysis without interfering with the regular growth and function of the cells (Eissner et al., 2002).
237 This study sought to determine whether Defibrotide, given in combination with MSC, would
238 increase the protection achieved by the MSC alone. In line with previous findings MSC
239 decreased the lysis of EC, notably however, the addition of defibrotide increased the significance
240 of inhibition, adding to the abrogation of lysis achieved by MSC alone (Fig. 4).

241 **Discussion**

242 The current study sought to examine the role of MSC in the protection of EC from CTL
243 induced lysis. Herein we have shown that MSC from multiple sources, not only bone marrow,
244 can abrogate EC cytotoxicity and work cooperatively with defibrotide to prevent EC death.
245 PGE2 was demonstrated to play a partial role in MSC protection with global inhibition of COX
246 signalling increasing EC lysis to levels similar to that of EC and CTL alone. To our knowledge
247 these finding are the first of their kind, establishing a role for MSC protection of EC from CTL
248 killing.

249 MSC influence over vascular cells has been studied in depth, in most cases their efficacy
250 is demonstrated in models of vascular pathophysiological diseases (Togel et al., 2007, Bader et
251 al., 2014, Sadat et al., 2007). Though much is yet unknown about their mechanism of action,
252 many such studies agree that the health of the endothelium is a major factor in the pathogeneses
253 of vascular diseases (Aird, 2003) and organ failure (Paris et al., 2001). Previous work by our
254 group into the role of CTL in EC cell lysis identified a sub population of $CD8^{+}CD28^{-}$ T
255 lymphocytes, that once activated target allogenic EC and induce cell lysis. The same study also
256 challenged the established dogma of Treg cells suppressing lymphocyte activity, showing that
257 the presence of Treg in this system enhanced the lytic activity of CTL (Eissner et al., 2011). This
258 was an important discovery as Treg are commonly studied as potential cellular therapeutic in the
259 treatment of GvHD (Edinger et al., 2003). Following on from this work, MSC were examined
260 herein for their ability to abrogate the MHC Class I restricted lysis of EC by CTL. There are two
261 possible routes for MSC to function in this system; through interaction with the HMEC 24 hours
262 prior to the cytotoxicity assay, or through their interaction with the CTL during the cytotoxicity
263 assay. Size exclusion assays using heart explants cells, similar in size to MSC, eliminated the

264 possibility that the MSC were simply masking the HMEC and thus interaction sites, while
265 animal studies, such as that by Togel *et al*., demonstrate how the release of trophic mediators by
266 MSC have a protective and regenerative effect on EC (Togel et al., 2007). In the context of MSC
267 inhibition of CTL, *in vitro* studies have concluded that MSC inhibit the proliferation and
268 cytotoxicity of CTL (Uccelli et al., 2008) without themselves being susceptible to CTL killing
269 (Rasmusson et al., 2007). Given the short exposure time between the MSC and CTL, it stands to
270 reason that the MSC directly affect the EC. It is interesting to note that cell-to-cell contact
271 between EC and MSC is required for their full protective efficacy, which is in contrast to studies
272 with mixed lymphocyte cultures where MSC could inhibit T cell proliferation even in a transwell
273 system (Weiss et al., 2008, Hartmann et al., 2010). However, evidence from inhibitor studies
274 herein suggest a role for PGE2 in EC protection (just like it can be seen in T cell inhibition
275 (Rossi et al., 2012, Hartmann et al., 2010)), yet levels of the prostaglandin increase where MSC
276 and CTL are in contact (data not shown). One hypothesis to explain these results is that of
277 licencing. MSC are known to react to the local milieu, a characteristic elegantly described by
278 Tobin *et al* where in the absence of prior licencing, MSC failed to improve the outcome of *in*
279 *vivo* GvHD following early administration (Tobin et al., 2013). In this instance, the interaction
280 between MSC and CTL may trigger an increase in PGE2 production resulting in protection of
281 EC. This hypothesis is further supported by the fact that when MSC in transwell experiments
282 failed to achieve the same level of inhibition. The clear abrogation of EC lysis by MSC
283 demonstrated in this study suggests that future adjuvant therapies may look to MSC as an
284 additional treatment option for solid organ rejection and GvHD, particularly in cases where the
285 endothelium is the therapeutic target and Treg cell treatment would be unsuitable.

286 The cooperative relationship between MSC and the endothelial stabilising drug
287 defibrotide demonstrated herein, speaks to their capacity as a potential prophylactic treatment in
288 host conditioning or organ transplantation. This study has also shown that the source of MSC
289 does not alter the protection provided. MSC from placental sources are known to be less
290 immunogenic than those isolated from the bone marrow (Rossi et al., 2012) and thus offer a
291 greater degree of immune privilege for 3rd party transplantation. They also show an increased
292 rate of proliferation allowing for a more efficient isolation (Pianta et al., 2014). Prevention is
293 always better than cure; we have shown that MSC therapy protects the endothelium from
294 targeted lysis, and if used in conjunction with Defibrotide can improve endothelial health,
295 reducing the risk of graft rejection. The health of the endothelium is key to the success of
296 transplant medicine and MSC are demonstrated herein to offer a tangible means of preventing
297 EC lysis from allogenic immune cells.

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308

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409 **Figure Legends**

410 **Figure 1. Allogeneic bone marrow MSC protect HMEC from CTL induced lysis.** $CD8^+$ T
411 cells (CTL) were stimulated with HMEC, treated with 50 μM Silibinin, for 7 days in the
412 presence of IL-2. Cytotoxicity assays were performed with HMEC cultured 24h previous in the
413 presence (green squares) or absence (red circles) of BM-MSC in the ratio of 5:1 HMEC to MSC.
414 Effector cells (CTL) were cultured with target cells (HMEC) in descending ratios of 20:1, 10:1
415 and 5:1 for 4 hours. EC lysis was determined by courter staining with propidium iodide (PI) and
416 analysed by flow cytometry (A). Results from multiple experiments were graphed using data
417 from the 20:1 ratio groups (B). n=12, $***P \leq 0.001$.

418 **Figure 2. Cell contact is required for MSC protection of EC in cytotoxicity assay.** CTL were
419 cultured with HMEC for 7 days. At day -1 $5X10^4$ HMEC were seeded in a 24 well plate, $1x10^4$
420 BM-MSC were added to the culture either in contact with the HMEC or in a transwell chamber.
421 The cells were cultured for 24h before addition of CTL to the 24 well plate, always in the lower
422 chamber, in contact with the HMEC. As before cells were cultured for 4 hours then
423 counterstained with PI and analysed by flow cytometry (A). PGE2 production was inhibited by
424 the addition of Indomethacin (40μM) to the HMEC and BM-MSC co-culture at day-1 (B).
425 Results from multiple experiments were graphed using data from the 20:1 ratio groups. n=3, $*P \leq$
426 0.05, $***P \leq 0.001$.

427 **Figure 3. Co-culture of HMEC with multiple sources of MSC result in protection from**
428 **CTL lysis.** CTL were cultured with HMEC for 7 days. At day -1 HMEC were co-cultured in the
429 presence or absence of BM-MSC, PVSC or hAMSC at a ratio of 5:1 respectively for 24h.

430 Targets were analysed for lysis as described in figure legend 1. Data from 4 independent
431 experiments with 4 different PBMC donors. *$P \leq 0.05$.

432 **Figure 4. Defibrotide adds to the protective effect of MSC.** CTL were cultured with HMEC
433 for 7 days. At day -1 HMEC were co-cultured in the presence or absence of BM-MSC at a ratio
434 of 5:1, with or without the addition of Defibrotide (25µM) for 24h. Targets were analysed for
435 lysis as described in figure legend 1. Results from multiple experiments were graphed using data
436 from the 20:1 ratio groups. n=3, *$P \leq 0.05$, **$P \leq 0.01$.

437 **Supplementary Figure 1. CTL lysis of HMEC is MHC Class I dependent.** CTL were
438 cultured with HMEC for 7 days. At day -1 HMEC were co-cultured in the presence or absence of
439 BM-MSC at a ratio of 5:1, with or without the addition of w6/32 for 24h. Targets were analysed
440 for lysis as described in figure legend 1. Results from multiple experiments were graphed using
441 data from the 20:1 ratio groups. n=3, *$P \leq 0.05$, **$P \leq 0.01$.

442 **Supplementary Figure 2. MSC size matched cells have no effect on EC lysis by CTL.** CTL
443 were cultured with HMEC for 7 days. At day -1 HMEC were co-cultured in the presence or
444 absence of BM-MSC or HH7 cells at a ratio of 5:1 for 24h. Targets were analysed for lysis as
445 described in figure legend 1. Results from multiple experiments were graphed using data from
446 the 20:1 ratio groups. n=3, *$P \leq 0.05$, **$P \leq 0.01$.

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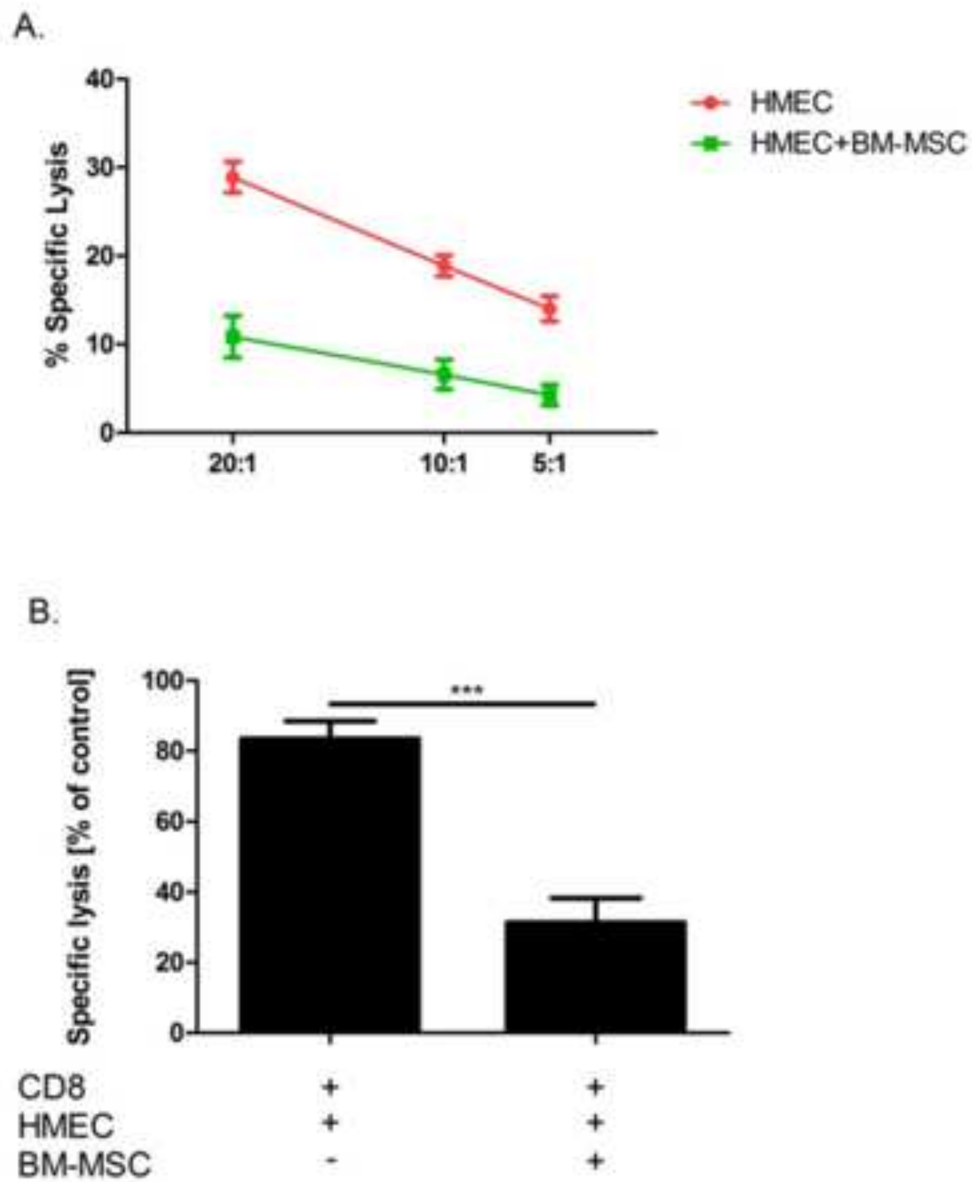
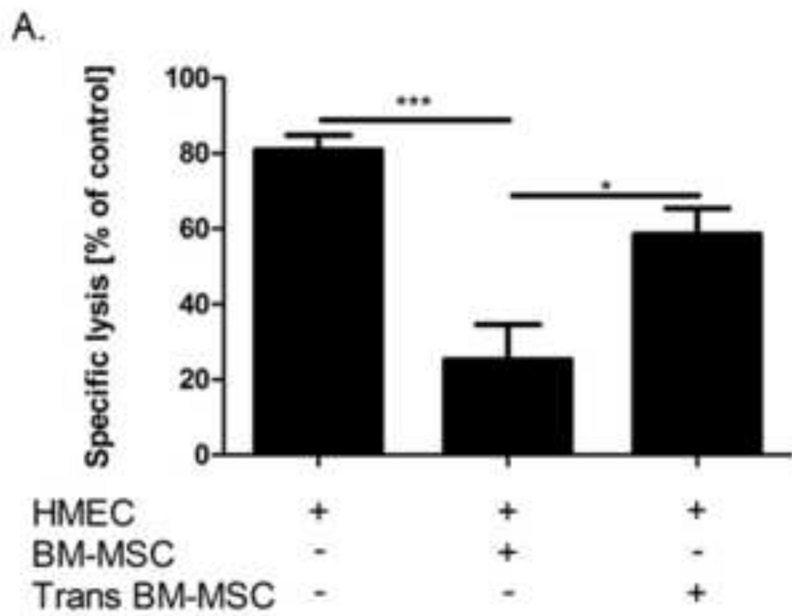


Figure 1



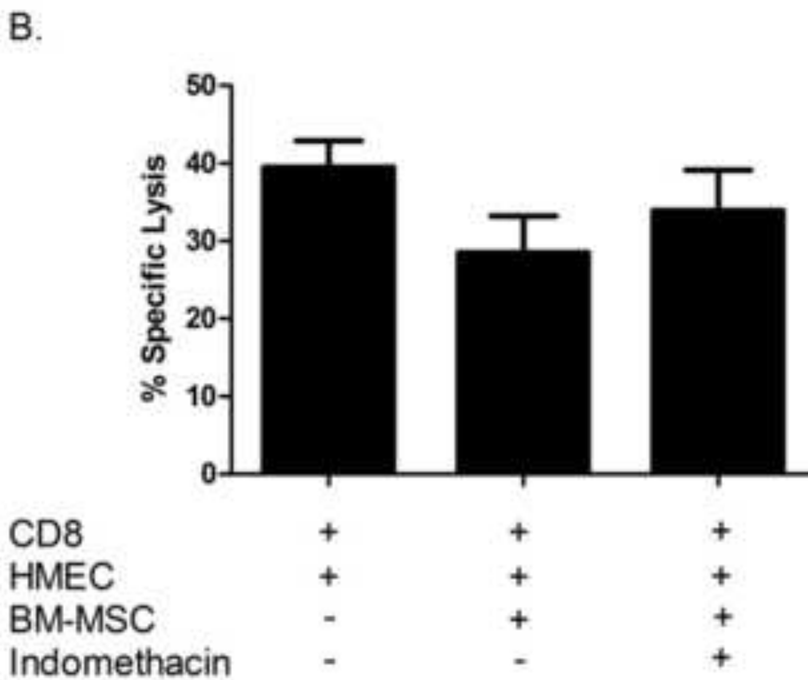
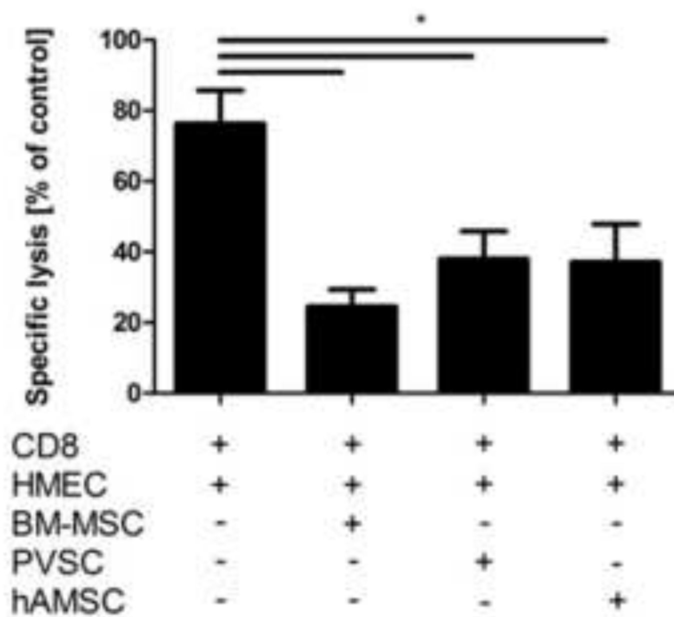


Figure 2



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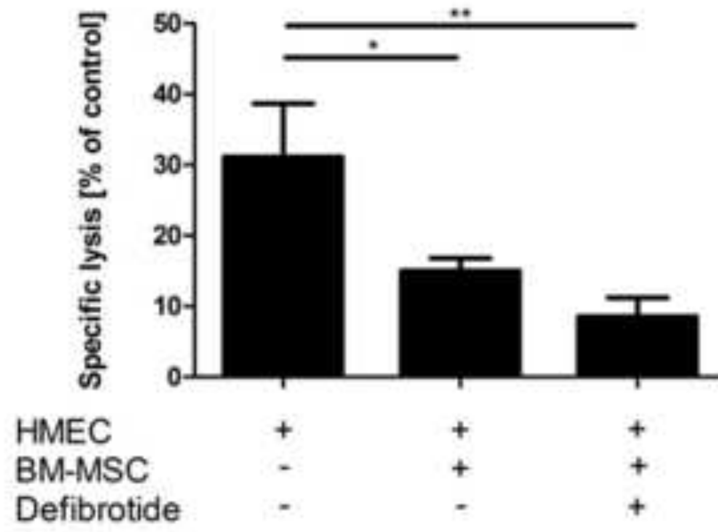

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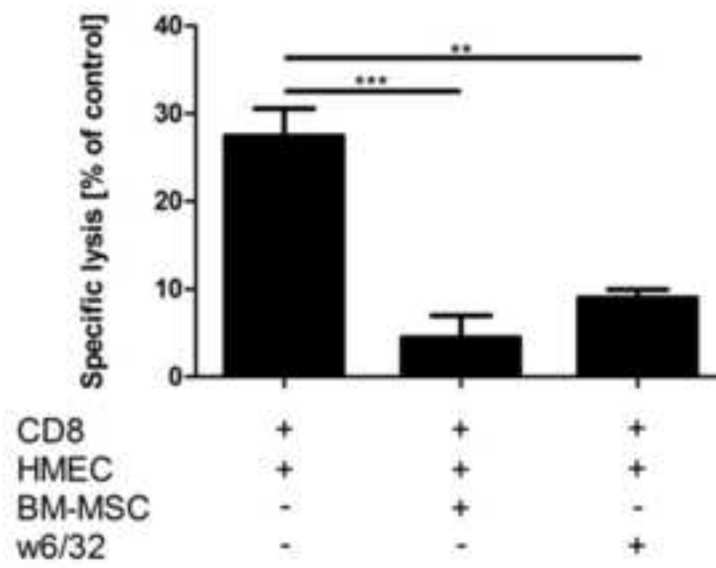
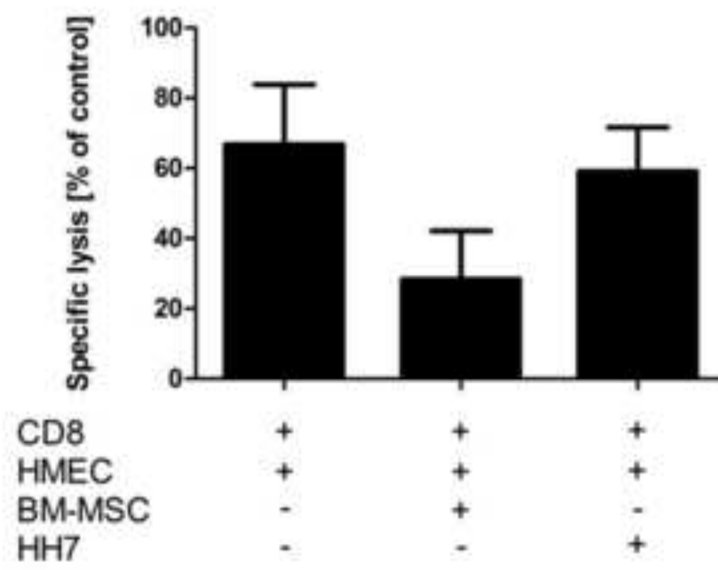


Supplementary
Figure 1



Supplementary
Figure 2