Are donor lymphocytes a barrier to transplantation tolerance?

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Abstract:

Purpose of review:

Following solid organ transplantation (SOT), populations of donor lymphocytes are frequently found in the recipient circulation. Their impact on host alloimmunity has long been debated but remains unclear, and it has been suggested that transferred donor lymphocytes may either promote tolerance to the graft, or hasten its rejection. We discuss possible mechanisms by which the interaction of donor passenger lymphocytes with recipient immune cells may either augment the host alloimmune response, or inhibit it.

Recent findings:

Recent work has highlighted that donor T lymphocytes are the most numerous of the donor leukocyte populations within a SOT and that these may be transferred to the recipient after transplantation. Surprisingly, graft-versus-host recognition of major histocompatibility class II on host B cells by transferred donor CD4 T cells can result in marked augmentation of host humoral alloimmunity and lead to early graft failure. Killing of donor CD4 T cells by host NK cells is critical in preventing this augmentation.

Summary:

The ability of passenger donor CD4 T cells to effect long-term augmentation of the host humoral alloimmune response raises the possibility that *ex-vivo* treatment or modification of the donor organ prior to implantation may improve long-term transplant outcomes.

Keywords: alloantibody, allorejection, donor lymphocytes, graft-versus-host disease,

tolerance, unlinked-help

Introduction

In solid organ transplantation (SOT), the delivery of passenger donor lymphocytes (DLs) to recipients is almost unavoidable. Passenger lymphocytes have been detected in the recipient's circulation within the first two hours following murine liver [1]; primate kidney [2]; and human liver transplantation [3,4]. The presence of DLs can be associated with graft versus host disease (GVHD), passenger lymphocyte syndrome, or may possibly induce tolerance. The impact of transfer of DLs will likely be determined by: the microenvironment of the transplanted organ; migration and sensitisation status of DLs; and the capacity for interaction with host immune constituents, as recently reviewed [5].

Organs such as the small intestines and lungs represent the first physiological mucosal defence barrier and possess large numbers of graft-resident lymphocytes. The generally poor long-term outcomes associated with these organs may be attributed to the simultaneous transfer of DLs contained within the allograft upon transplantation [6]. Tissue-resident lymphocytes have been a particular research focus recently, but the role of this subset in SOT has yet to be clarified. This review will first describe the make-up of the tissue-resident lymphocyte populations that are likely to be present within an allograft. We will then discuss how these populations may contribute to graft tolerance and rejection, before considering how strategies that specifically target DLs may be used to prolong graft survival.

Passenger lymphocytes populations within an allograft.

The nature of tissue-resident lymphocytes differs in lineage and phenotype and includes B cells [7], CD8+ [8,9], FoxP3+ [10,11], innate γδ [12,13], NK T cells [14,15] and CD4 T cells [16]. These have been reported in various tissues such as the skin, gut, lungs, kidney, and liver. To what extent these populations remain resident within the allograft or egress to the recipient's

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circulation is not yet known, but is clearly an important distinction – those DLs that migrate to the recipient's secondary lymphoid tissue will presumably have greater influence on host alloimmunity. In this respect, this review will focus on donor B cells and CD4+ T cells, because their potential for migration and interaction with host immune constituents suggests the greatest capacity for shaping the alloimmune response.

Passenger donor B lymphocytes in allograft rejection and tolerance.

Although not as prominent feature as T lymphocytes, circulating donor –strain B lymphocytes (dnB cells) are frequently detectable in recipients of solid organ transplants, particularly following liver and intestinal transplantation [17]. Appreciable numbers are also released into the circuit during ex vivo normothermic perfusion of porcine kidneys [18]. How such transfer impacts upon the host alloimmune response remains unclear. Most immediately, migrating dnB cells will deliver MHC class I and class II alloantigen, and could potentially prime cytotoxic CD8 T cell and direct-pathway CD4 T cell alloresponses in the recipient. However, resting B cells do not generally express sufficient co-stimulatory ligands for activation of naïve T cells [19-21], and indeed, have been targeted as a possible therapeutic strategy in autoimmunity [22], raising the possibility that transferred dnB cells may inhibit the host response against the graft. Against this, activated [23,24] or memory [25,26] B cells are effective antigen presenting cells for driving naïve T cell activation, and it seems likely that even if transferred as naïve cells, exposure to previously unencountered recipient antigen within the inflammatory environment of the graft [27], will lead to full B cell activation. In support, recent murine studies have reported a positive role for passenger dnB cells in triggering recipient CD4 T cell alloimmunity [28], although the impact was relatively modest when compared with the contribution from transferred donor dendritic cells.

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One striking manifestation of the transfer of dnB cells is Passenger Lymphocyte Syndrome, in which haemolysis is triggered by donor B cell recognition of mismatched ABO blood group antigens in the recipient [29]. Whether this relates to transfer of plasma cells or to B cells that undergo further differentiation to antibody secretors within the recipient is not known, but the latter is suggested by a recent report highlighting similar donor-derived antibody profiles in a pair of recipients from the same deceased kidney donor [30]. The recipient antibody responses observed with Passenger Lymphocyte Syndrome appear transient, presumably because transferred donor plasma cells are not deposited in an appropriate recipient niche to facilitate long-term survival, and it is therefore doubtful that they contribute to allograft rejection, particularly because transfer of responsiveness against donor human leukocyte antigen HLA antigen would be highly unusual.

Several recent clinical studies have suggested that a state of operational tolerance is associated with a signature B cell phenotype [31-33], with an expanded transitional B cell population. Although suppressor function has not been confirmed in the clinical setting, this is consistent with development of regulatory B cells [34], and B cells have been shown to be important mediators of tolerance in murine transplant models [35]. It is generally assumed that the putative regulatory B cell population is of recipient origin, and is seems unlikely, given their presentation of mismatched MHC class I and class II alloantigens, that donor B cells would survive sufficiently long in the recipient to provide regulatory function. However, although poorly understood, regulatory B cell development is favoured by an inflammatory environment [36,37], and requires positive signalling via the B cell receptor, CD40 and TLR ligation [38]. Thus although not yet demonstrated, Interactions with host T cells and antigen presenting cells could conceivably provide the appropriate triggers for differentiation of

migrating passenger donor B lymphocytes into regulatory cells that promote allograft survival.

Passenger donor T lymphocytes in allograft rejection and tolerance

The most abundant lymphocyte subset contained within a SOT is likely to be the CD8 and CD4 T cell [16,18]. Although these may be naïve circulating T cells that are trapped within the graft microcirculation, additional populations of memory lymphocytes within the graft parenchyma (so called tissue resident memory (T_{RM}) lymphocytes) are also likely to be transferred ([5,39]). Transfer of donor T lymphocytes (dnT cells) is most readily manifest by the development of graft versus host disease in the recipient[40,41].

One might anticipate that GVH responses mediated by dnT cell transfer would favour enhanced allograft survival, by inhibiting host alloreactivity through cytotoxic destruction of host antigen presenting cells and alloreactive lymphocyte subsets. Our studies of mouse chronic heart allograft rejection however highlighted that in the MHC class II mismatched 'bm12' to C57BL/6 model, a key component of the rejection response was the triggering of recipient anti-nuclear autoantibody responses [42,43]. These responses were class-switched, but surprisingly, T cell help for their initiation was provided, not by recipient CD4 T cells, but by donor CD4 T cells. The potential contribution of autoimmunity to allograft rejection is increasingly emphasised (reviewed in [44-46]) and T cell depletion experiments suggested a direct role for the autoantibody response observed in our model in mediating progression of allograft vasculopathy [42,47].

Autoantibody responses did not develop in mice that selectively lacked MHC class II expression only on B cells, highlighting that cognate interaction between the dnCD4 T cell and host B cell was the direct trigger for initiating humoral autoimmunity. This raises the

question as to the precise MHC II / peptide complex that was recognised on the surface of the host B cells, and in this regard it was notable that the entire follicular B cell population up-regulated MHC class II and costimulatory ligand expression, in keeping with global activation[47]. Further experiments incorporating T cell deficient recipients highlighted that the critical step for recipient B cells differentiation to an antibody-secreting cell was concurrent BCR ligation with target antigen[47]. This reflects the peculiarities of direct-pathway allorecognition, in which as much as 5% of an individual's T cell repertoire recognises intact MHC alloantigen [48-50]. Consequently, the dnCD4 T cell fraction is likely to recognise the majority, if not all, MHC class II complexes on host B cells, irrespective of the particular peptide bound in the binding groove. Thus, the dnCD4 T cells provide permissive help to all B cells; antibody secretion is determined instead by availability of target antigen (figure 1a). Presumably, the autoantibody responses observed in our experiments reflect activation of anergic autoreactive B cells that are already bound via their BCR to target autoantigen.

An intriguing possibility raised by this unusual, 'peptide-degenerate' help is that dnCD4 T cells can provide help to recipient B cells for the production of alloantibody against alloantigenic determinants expressed on the surface of the dnCD4 T cell (figure 1b). This occurs despite those CD4 T cells clearly being tolerant to that antigen when encountered restricted to self in the donor [51]. We further showed that the provoked alloantibody response resulted in rapid lysis of dnCD4 T cell fraction; effectively the dnCD4 T cells actively promote their own destruction. This further suggests that passenger CD4 T cells within the transplant could contribute to graft rejection. This was tested by developing a murine model of chronic heart allograft rejection in which the donor 'bm12.Kd.IE' strain differed from the

C57BL/6 recipient at additional MHC class I (H-2K^d) and class II (I-E^d and I-A^{bm12}) loci. Heart grafts were rejected slowly, with the development of progressive allograft vasculopathy that was associated with robust alloantibody responses directed against the mismatched class I and class II alloantigens. Critically, alloantibody responses were markedly reduced in recipients of heart grafts from T-cell depleted donors, as were recipient cytotoxic CD8 T cell alloimmune and indirect-pathway CD4 T cell responses. Heart allografts from T cell deficient donors exhibited markedly reduced allograft vasculopathy [51], thus confirming an important role for passenger dnCD4 T cells in augmenting host alloimmunity.

Although these findings would appear to shift emphasis from the recipient alloreactive CD4 T cell population as the central mediator of allograft rejection, it was notable that rejection was still dependent upon the recipient CD4 T cell fraction. Our ongoing work suggests that recipient CD4 T cells are required for provision of essential T follicular helper cell function that maintains long-lasting germinal centre alloantibody responses: although dnCD4 T cells could still trigger recipient humoral immunity in T cell deficient recipients, germinal centre responses did not develop and heart grafts survived indefinitely without development of allograft vasculopathy[47]. Thus optimal recipient effector humoral responses required collaboration between donor and recipient CD4 T cell fractions (figure 1c).

The model we employed was undoubtedly developed to facilitate examination of the potential contribution of dnCD4 T cells to graft rejection, and could be justly criticised as lacking immediate clinical relevance. What therefore are the likely implications of our findings to clinical transplantation? First, our models were characterised by relatively limited MHC mismatch between the donor and recipient and the ability of donor CD4 T cells to interact productively with the recipient B cell population was dependent upon avoidance of

rapid destruction by host NK cell recognition. Notably, in a completely MHC-mismatched (BALB/c to C57BL/6) model of chronic alloantibody mediated rejection, augmentation of the host alloantibody response by passenger CD4 T cells only occurred upon depletion of the recipient NK cell population. Such depletion resulted in rapid (acute) graft rejection [51]. The role of host NK cells in graft rejection remains controversial [52], and our findings highlight an important, and previously unappreciated, role for NK cells in preventing graft rejection through recognition of donor passenger lymphocytes. Killer cell Immunoglobulin-like receptor (KIR) recognition is complex and still only partly understood [53], but current MHC matching practices in clinical renal transplantation will result in approximately half of donor – recipient combinations being matched at KIR loci [54], thereby enabling donor passenger lymphocytes to avoid rapid NK-cell mediated destruction. Whether this results in poorer long-term allograft survival is not known, because studies that have examined the role of NK cells in clinical graft rejection have generally studied MHC-matched donor / recipient combinations to avoid the confounding impact of adaptive HLA allorecognition[54,55] whereas the impact of NK cell recognition observed in our model is dependent upon donor / recipient MHC class II mismatching. Avoidance of NK cell killing does not, however, guarantee long-term survival of the donor CD4 T cell fraction; as mentioned above, the donor CD4 T cell fraction was still killed rapidly (within one week of transplantation) by the adaptive alloimmune responses that they provoke in the host. This illustrates that short-lived immune interactions that occur in the peri-transplant period may have long-lasting consequences; despite their rapid destruction, the impact of donor CD4 T cells on augmenting host humoral alloimmunity was evident many weeks after transplantation.

Most solid organs are now known to harbour populations of resident memory T (T_{RM}) lymphocytes (reviewed in [56]), which are phenotypically distinct from the central and effector memory subsets. Donor-derived T_{RM} lymphocytes will presumably be transferred within most solid organ allografts, yet their contribution to host alloimmunity is not known [5, 39]. Although our experiments did not distinguish between T_{RM} lymphocytes within graft parenchyma and naïve circulating T cells caught in the graft microcirculation, they do nevertheless suggest a potential mechanism by which transferred donor T_{RM} cells may influence the host alloimmune response. In this respect, it was notable that heart allografts from donors that had been primed six weeks earlier by a recipient strain skin graft (thus generating anti-recipient memory responses) provoked stronger alloantibody responses and were rejected much more rapidly than heart grafts from naïve donors [47,51].

Our results suggest that strategies that deplete passenger donor lymphocytes within the allograft may hold potential to prolong allograft survival. This could possibly be achieved by performing *ex vivo* normothermic perfusion of the organ after retrieval; an approach that is gaining popularity because of its potential to 'recondition' the organ and improve viability [57]. Early reports suggest that substantial numbers of donor T lymphocytes are recovered from the circuit during the *ex-vivo* perfusion phase [18], and that would otherwise be released into the recipient's circulation.

Before such strategies are attempted, however, it is worth considering whether resident donor lymphocytes could provide protective function and promote graft survival. This has not been studied, but mucosal T_{RM} cells provide an important first-line defence against tissue re-infection, and are likely involved in maintaining cytomegalovirus (CMV) latency [58-60]. Deletion of donor TRM within an allograft could therefore possibly exacerbate the

intragraft inflammatory milieu due to CMV re-activation, which may in turn provoke host alloimmunity and increase the incidence of allograft rejection [61].

In addition to T_{RM} lymphocytes, there is increasing evidence supporting a distinct population of tissue-resident regulatory T cells [62]. These are thought to regulate local responses within the organ and suppress development of autoimmunity – T cell receptor sequencing suggests skewing towards recognition of self-antigen [63,64]. Persistence of tissue-resident donor regulatory T cells after transplantation could conceivably favour allograft survival, through, for example, production of suppressor cytokines, such as IL-10 and TGF-β, that promote an anti-inflammatory environment within the allograft. This has not been tested, but resonates with the growing appreciation that local immune events within the graft shape the recipient's alloimmune response [65,66], and parallels the observation that recipient regulatory T cells mediate suppression principally within the allograft [67,68].

Finally, similar to the peptide-degenerate, graft-versus-host recognition of recipient MHC class II complexes by donor-strain CD4 T effector cells, passenger donor CD4 T regulatory cells may also interact with a large proportion of the recipient B cell population. This interaction would instead, however, be expected to profoundly inhibit host humoral immunity (figure 1d). In support, bm12 heart allografts from donors that have been depleted of regulatory T cells (by administering anti-CD25 mAb to the bm12 donor prior to heart allograft rejection) are rejected much more rapidly by C56BL/6 recipients than heart grafts from unmodified bm12 donors (unpublished data, GJP).

Conclusions

Many different subsets of donor lymphocytes are likely to be transferred to the recipient within a solid allograft. *Ex vivo* perfusion studies suggest that the T lymphocyte subset is the

most numerous and is readily released into the recipient's circulation. Whether this reflects

release of circulating T cells that have been trapped within the graft microcirculation or

mobilisation of T$_{RM}$ cells from graft parenchyma has yet to be determined, but is likely an

important distinction. Our work has highlighted one potential mechanism by which donor

CD4 T cell interaction with the recipient B cell population augments the host alloimmune

response, as most dramatically demonstrated by the accelerated rejection of murine heart

allografts from donors that have primed against recipient. A testable clinical consequence of

this observation is that kidney transplants from living donors sensitised against recipient

(typically mother to offspring) may be associated with higher rates of rejection than normal.

Key points

- **Transfer of passenger donor lymphocytes is common after solid organ transplantation**
- **Transferred passenger donor CD4 T cells can potentially interact productively with recipient B cells for long-lasting augmentation of the host alloimmune response.**
- **This augmentation is more pronounced if memory donor CD4 T cells with specificity for recipient are transferred.**
- **Host NK cell recognition and destruction of passenger lymphocytes is critical for preventing this interaction between donor and recipient lymphocytes.**
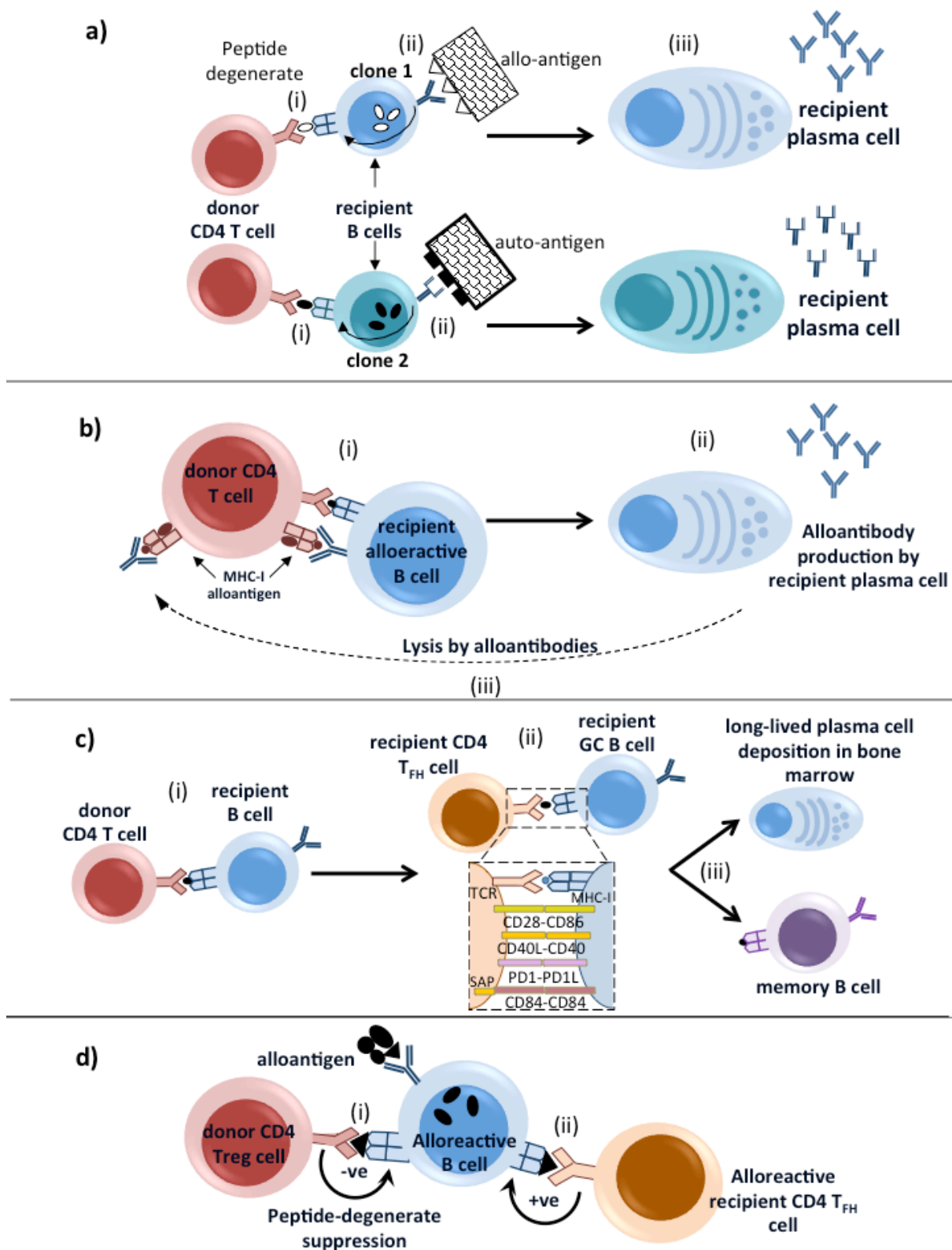
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None

Conflicts of interest

The authors declare no conflict of interest

Figure 1

 Heading: **Peptide-degenerate interactions between donor CD4 T cells an recipient B cells.**

Legend: a) *Top panel*: **i)** Donor CD4 T cells TCR recognise the majority of, if not all, MHC class II complexes expressed on recipient B cells as foreign, regardless of the loaded peptide. This 'peptide-degenerate' recognition results in global activation of all recipient B cells. Recipient B cells differentiation into antibody-secreting plasma cells is, however, determined by the concurrent BCR ligation to target antigen; hence, different antibody specificities are generated depending upon antigen availability. This includes recognition of self antigen for development of autoantibody **(ii)**.

b) Donor CD4+ T cells expressing alloantigenic determinants can provide help to recipient B cells for production of alloantibody directed against alloantigen determinants expressed on the donor T cells. This alloantibody can, in turn, result in lysis of the donor CD4 T cells.

c) Although donor CD4 T cells trigger recipient B cell activation and plasma cell differentiation, germinal center responses are not observed unless secondary involvement of a population of recipient-derived T follicular helper T cells. This potentially results in deposition of high-affinity long-lived plasma cells in bone marrow and generation of memory B cells. PD-1, programmed death-1;; PD-1L, programmed death-1 ligand; SAP, SLAM-associated protein.

d) Peptide-degenerate suppression by passenger donor regulatory CD4 T cells may inhibit alloreactive B cell activation, by counteracting positive 'peptide-specific'' signalling provided by alloreactive recipient CD4 helper T cells. BCR, B cell receptor; MHC, major histocompatibility complex; SLAM, Signaling lymphocytic activation molecule; TCR, T cell receptor.

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