

Immunosuppressive Property of Dried Human Amniotic Membrane

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Key Words

Amniotic membrane · T cell proliferation · Immune suppression · Cryopreservation · Ocular surface

Abstract

Purpose: To report the immunosuppressive property of dry human amniotic membrane (dHAM). **Methods:** Mouse splenocytes harvested from Balb/c mice were stimulated using functional-grade anti-CD3e antibodies for 4 days either with or without either of 2 commercial types of dHAM (Ambiodry 1 & 2, IOP Inc., Costa Mesa, Calif., USA) added to the culture media. The cell proliferation assay was performed to analyze the extent of splenocyte proliferation. **Results:** dHAMs significantly suppressed mouse splenocyte proliferation compared to control. The suppression by dHAM with intact amniotic epithelium (Ambiodry 2) was significantly stronger than dHAM without epithelium (Ambiodry 1). **Conclusion:** The immunosuppressive property of dHAM was demonstrated using an alloreactive splenocyte proliferation assay.

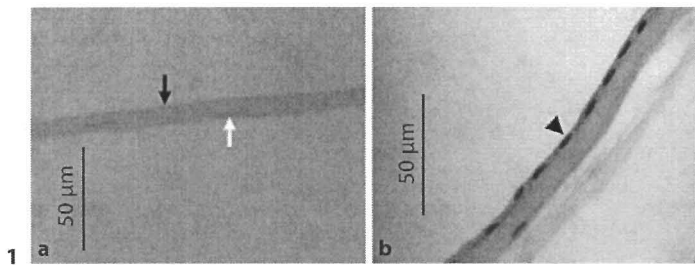
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Human amniotic membranes are widely used in the treatment of various pathologic ocular surface conditions [1]. Although the immunosuppressive properties of cryopreserved membrane have been studied before [2], we are unaware of previous reports describing the immunologic properties of dry HAM (dHAM). Separate evaluation of the functional properties of dHAM is necessary because of its distinctly different preparation process. In contrast

to cryopreserved human amniotic membrane, dHAM is low-electron-beam sterilized (18–20 kGy) and preserved using low heat and air vacuum [3]. Currently, two types of dHAMs are commercially available: dHAM without amniotic membrane epithelium (Ambiodry 1) and dHAM with an intact monolayer of epithelium (Ambiodry 2). In this study, we evaluated the histological differences between these two types of dHAMs and the effect of dHAMs on mouse splenocyte proliferation stimulated by functional anti-CD3 antibodies.

All animal procedures were conducted in accordance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. Spleens were harvested from fresh-sacrificed, 15-week-old, Balb/c mice. Splenocytes were collected after lysis of red blood cells using RBC lysis buffer (eBiosciences, San Diego, Calif., USA). Fifteen wells of a single 96-well plate were coated with functional-grade anti-mouse CD3e antibodies (eBiosciences) 1 day before splenocyte application and another 5 wells were used as control without antibody coating. The splenocytes (5×10^5 cells per well) were plated in each well ($n = 20$) and a small piece (2×2 mm) of dHAM (Ambiodry 1 or 2, OKTO Ophtho Inc., Costa Mesa, Calif., USA) was added to 5 study wells each ($n = 10$). After 4 days of incubation, the 3-(4,5-dimethylthiazolyl-2)-2,5-diphenyltetrazolium bromide (MTT) assay (Cell Growth Determination Kit, MTT based, Sigma, St. Louis, Mo., USA) was performed according to the manufacturer's in-

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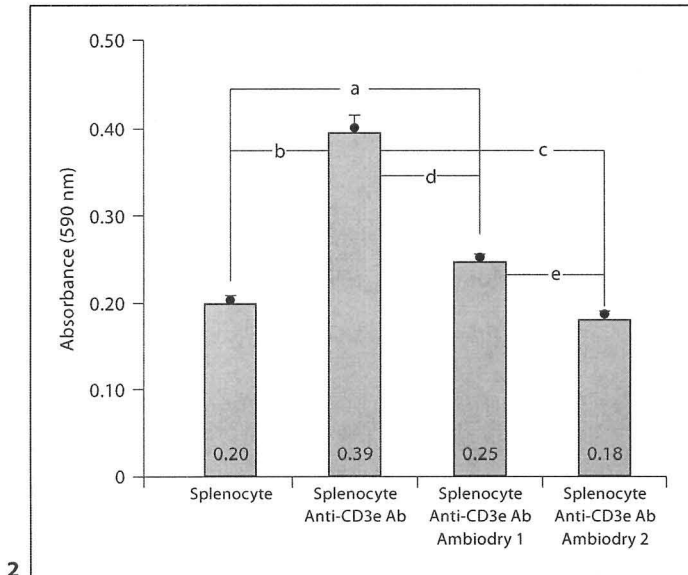


Fig. 1. Histology of dry human amniotic membrane. Hematoxylin and eosin staining of Ambiodry 1 (a) showed even thickness of stroma containing several nuclei of stromal cells (white arrow). The membrane lacks an epithelial layer. However, it has a well-preserved basement membrane layer (black arrow). Another type of dHAM (Ambiodry 2) (b) showed a well-preserved monolayer of amniotic membrane epithelium (arrowhead) attached to the underlying basement membrane and stroma. The stromal thickness is similar between both types of dHAMs.

Fig. 2. MTT assay of mouse (Balb/c) splenocyte proliferation. Adding dHAMs to the culture media (Ambiodry 1 or 2) successfully inhibited splenocyte proliferation. The suppression by Ambiodry 2 was significantly stronger than that by Ambiodry 1 and completely eliminated the proliferative response. Each bar stands for the average of 5 independent reactions (means \pm SEM). Statistical analysis was performed with the Kruskal-Wallis test and the least significant difference test using ranks for multiple comparisons. ^a $p = 0.032$; ^b $p < 0.001$; ^c $p < 0.001$; ^d $p = 0.34$; ^e $p < 0.001$.

structions. The absorbance was measured at 590 nm using a standard plate reader (Synergy[®] HT, Biotek, Winoski, Vt., USA).

On histologic examination, Ambiodry 1 lacked an epithelial layer and consisted of an even thickness of stroma in contrast to Ambiodry 2, which had a well-preserved

monolayer of amniotic membrane epithelium (fig. 1). Both types of dHAMs significantly decreased mouse splenocyte proliferation in response to anti-mouse CD3e antibodies, and Ambiodry 2 did so to a significantly greater extent (fig. 2).

Human amniotic membrane has been reported to produce various immunosuppressive cytokines such as interleukin 4, interleukin 10, transforming growth factor- β_1 and - β_2 [4–6]. Moreover, supernatant from amniotic epithelial culture has been reported to suppress inflammation [7]. Although the exact mechanism is still unclear, mouse T cell suppression has been previously demonstrated using cryopreserved amniotic membrane [2]. dHAMs are manufactured by a dehydration process using low heat; they lack viable cells; Ambiodry 1 lacks an epithelial layer. Although the immunosuppressive properties of cryopreserved amniotic membrane can be obviated by ethanol devitalization [2], our study demonstrates that the devitalized dry preparation of human amniotic membrane maintained its immunosuppressive property and that this characteristic was potentiated by the preservation of the amniotic epithelium. Thus, both amniotic membrane epithelium and stroma appear to be important for achieving optimal immunosuppression by amniotic membrane.

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