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Retinal pigment epithelial cells upregulate expression of complement factors after co-culture with activated T cells

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ABSTRACT

In this study we examined the effect of T cell-derived cytokines on retinal pigment epithelial (RPE) cells with respect to expression of complement components. We used an in vitro co-culture system in which CD3/CD28-activated human T cells were separated from the human RPE cell line (ARPE-19) by a membrane. Differential gene expression in the RPE cells of complement factor genes was identified using gene arrays, and selected gene transcripts were validated by q-RT-PCR. Protein expression was determined by ELISA and immunoblotting. Co-culture with activated T cells increased RPE mRNA and/or protein expression of complement components C3, factors B, H, H-like 1, CD46, CD55, CD59, and clusterin, in a dose-dependent manner. Soluble factors derived from activated T cells are capable of increasing expression of complement components in RPE cells. This is important for the further understanding of inflammatory ocular diseases such as uveitis and age-related macular degeneration.

1. Introduction

Interactions between the eye and the immune system lie at the heart of many ocular diseases such as uveitis, diabetic retinopathy, and age-related macular degeneration (Bora et al., 2008; Jha et al., 2007). In steady state, the ocular tissues provide immunosuppressive signals to maintain the ocular immune privilege that is important for normal vision. However, in order to protect the eye from infection, ocular tissues also express inflammatory factors, e.g. proteins from the complement system.

The complement system can be activated through three pathways; the classical, the lectin, and the alternative, which converge by the formation of a C3 convertase. This ultimately results in the formation of the membrane attack complex (C5b-9). Although complement is considered a branch of the innate immune system, it is also implicated in the regulation of the adaptive immune system, through activation of T, B, and dendritic cells by C3b opsonization and release of the anaphylatoxins C3a and C5a (Strainic et al., 2008; Walport, 2001). This complex system is therefore very important for tissue homeostasis, including phagocytic removal of apoptotic cells and debris, and attraction of immune cells, and is under tight regulation by soluble and membrane-bound inhibitors (Fig. 5).

Many studies have addressed the regulatory effect of retinal pigment epithelial (RPE) cells on T cells. These studies have revealed roles of the prostaglandin (PG)E_2/thrombospondin (TSP)-1/TGFβ-axis with induction of T regulatory cells (Futagami et al., 2007; Liversidge et al., 1993; Sugita et al., 2006; Zamiri et al., 2005), glucocorticoid-induced tumor necrosis factor receptor family-related (GITR) and its ligand GITRL (Mahesh et al., 2006), galectin-1 (Ishida et al., 2003), and programmed death 1 (PD1) and its ligands B7H1 and B7DC (Sugita et al., 2009; Usui et al., 2008). In addition, several groups have investigated the effect of immune cells (T cells, monocytes), cytokines (IFNγ, TNFα, IL1β and IL6), and other inflammatory mediators (LPS, poly I:C) on RPE cells. However, most studies have focused on a single or a few cytokines, chemokines, or molecules from the complement system (for review see Holtkamp et al., 2001). To our knowledge, no studies have investigated the entire complement pathway, or the effect of T cell co-culture on RPE cell complement expression.

Recent evidence supports that the complement system might be important in relation to T cell-mediated inflammation in the central nervous system and in ocular tissues, either by direct involvement or

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by augmenting the inflammatory process. Inflammation in general, and complement activation in particular, is suspected to play a role in several ocular diseases, including uveitis, diabetic retinopathy, and age-related macular degeneration (AMD) (Jha et al., 2007). Therefore, we decided to study the complement response in RPE cells exposed to inflammatory stress. We have used a co-culture system of the cell line ARPE-19 separated by a membrane from freshly purified, in vitro activated human T cells, in an effort to reflect T cell-mediated inflammatory conditions in the retina.

2. Materials and methods

2.1. Ethics statement

Ethical approval was waived by "Den Videnskabsetiske Komité Københavns og Frederiksberg Kommuner" which deemed that no results obtained in the experiments were related to the donors, and no samples or cells were stored; samples were identified by date of blood sampling only, and analyzed anonymously. Verbal consent to blood sampling was considered adequate by the Ethics Committee, and was obtained.

2.2. Cell culture

The adult human RPE cell line ARPE-19 (American Type Culture Collection) was cultured in 6-well cell culture plates at 37 °C with 10% CO_2 > 6 weeks, until pigmented monolayers had formed (Fig. 1). Cells were initially cultured in Dulbecco's modified Eagle's medium with 10% fetal calf serum (Lonza BioWhittaker), 300 μg/ml L-glutamine (Gibco), 50 μg/ml gentamicin (Gibco), and 2,5 μg/ml amphotericin B (Gibco). 2–4 weeks before experiments, culture medium was gradually changed to X-vivo 15 serum-free medium (Lonza BioWhittaker) with 300 μg/ml L-glutamine, 100 μg/ml penicillin and 100 μg/ml streptomycin, and 2,5 μg/ml amphotericin B.

2.3. T cell purification

T cells were purified from fresh whole blood from healthy, young volunteers, using three consecutive steps: (1) density-gradient centrifugation, (2) plastic adherence, and (3) antibody-based negative selection. Peripheral blood mononuclear cells (PBMCs) were isolated using Lymphoprep™ (Axis-Shield) in accordance with the manufacturer's instructions. PBMCs were enriched for T cells by 1.5 h incubation in T flasks at 37 °C, by which monocytes and B cells adhere to the plastic. The remaining non-T cells were depleted using the T cell Negative Selection kit from Dynal (Invitrogen). T cell purity was >90% (CD3 flow cytometric analyses; data not shown). In some experiments, $CD4^+$ or $CD8^+$ T cells were purified separately using the CD4 or CD8 Negative Isolation Kits from Dynal (Invitrogen).

2.4. RPE:T cell co-culture

T cells were cultured in Anopore™ 0.2 μm membrane inserts (Nunc) over RPE cells in the ratio 2.5 T cells: 1 RPE cell. In some experiments the ratio was lowered to 1 T cell: 4 RPE cells, 1:40, or 1:400. T cells were activated using Dynabeads® CD3/CD28 T Cell Expander (Invitrogen), and cells were co-cultured for 48 h at 37 °C with 5% CO_2. In some experiments culture time was reduced to 12 or 24 h. In other experiments RPE cells were grown on the inserts and T cells added to the bottom compartment. Because previous experiments indicated that the inserts themselves stimulated T cell proliferation, and that this stimulation was eliminated by the presence of serum, experiments with resting T cells and appropriate controls were performed with 10% human AB serum (Valley Biomedical) added to the culture medium. At the end of co-culture, media was collected, and RPE cells were removed using a cell scraper. RNA was purified using the NucleoSpin® RNA II kit (Macherey-Nagel) in accordance with manufacturer's instructions, or cells were lysed in lysis buffer (50 mM Tris–HCl pH 7.4, 150 mM NaCl, 1% Nonidet-P40, 0.25% Na-deoxycholate, 1 mM EDTA, 1 mM PMSF, 1 μg/ml aprotinin, 1 μg/ml leupeptin, 1 μg/ml pepstatin) for protein studies.

2.5. Microarrays

Two different oligonucleotide arrays were used: the genome-wide Human Genome U133 Plus 2.0 (Affymetrix, hereafter referred to as Affymetrix GeneChips) and the pathway-specific Oligo GEArray® OHS810 (SA Biosciences, hereafter referred to as GEArray), including 440 immunology-related genes.

Affymetrix GeneChips ($n = 2$/group) were processed at the Copenhagen University Hospital Microarray Center. Labeling of RNA samples was done according to Affymetrix protocol and all samples were scanned on the same scanner. Hybridization cel files were pre-processed using the MAS5 algorithm as implemented in Bioconductor (R Development Core Team, 2008). This algorithm converts the files obtained from scanning the microarrays into a set of expression values for each gene. Implemented in the process is also a normalization step that adjusts the expression levels from each sample. The pre-processing results in a spreadsheet of expression values for all probe sets and samples. Among the identified differentially expressed genes were several complement genes, and the complement system was chosen for further investigation. Probe sets

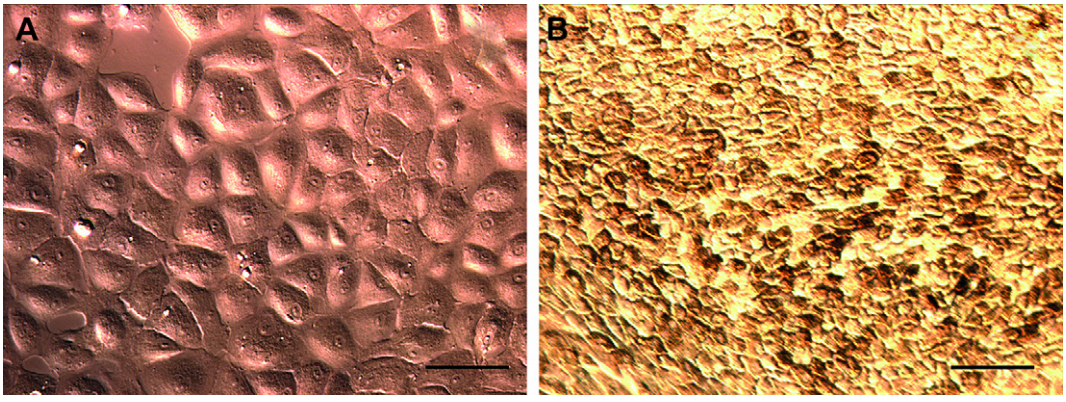

Fig. 1. ARPE-19 cells. (A) Unpigmented, fibroblast-like appearance of nearly confluent ARPE-19 cells 1 week after plating. (B) Pigmented, cobblestone-like appearance of densely packed ARPE-19 cells >6 weeks after plating. Bar = 50 μm.