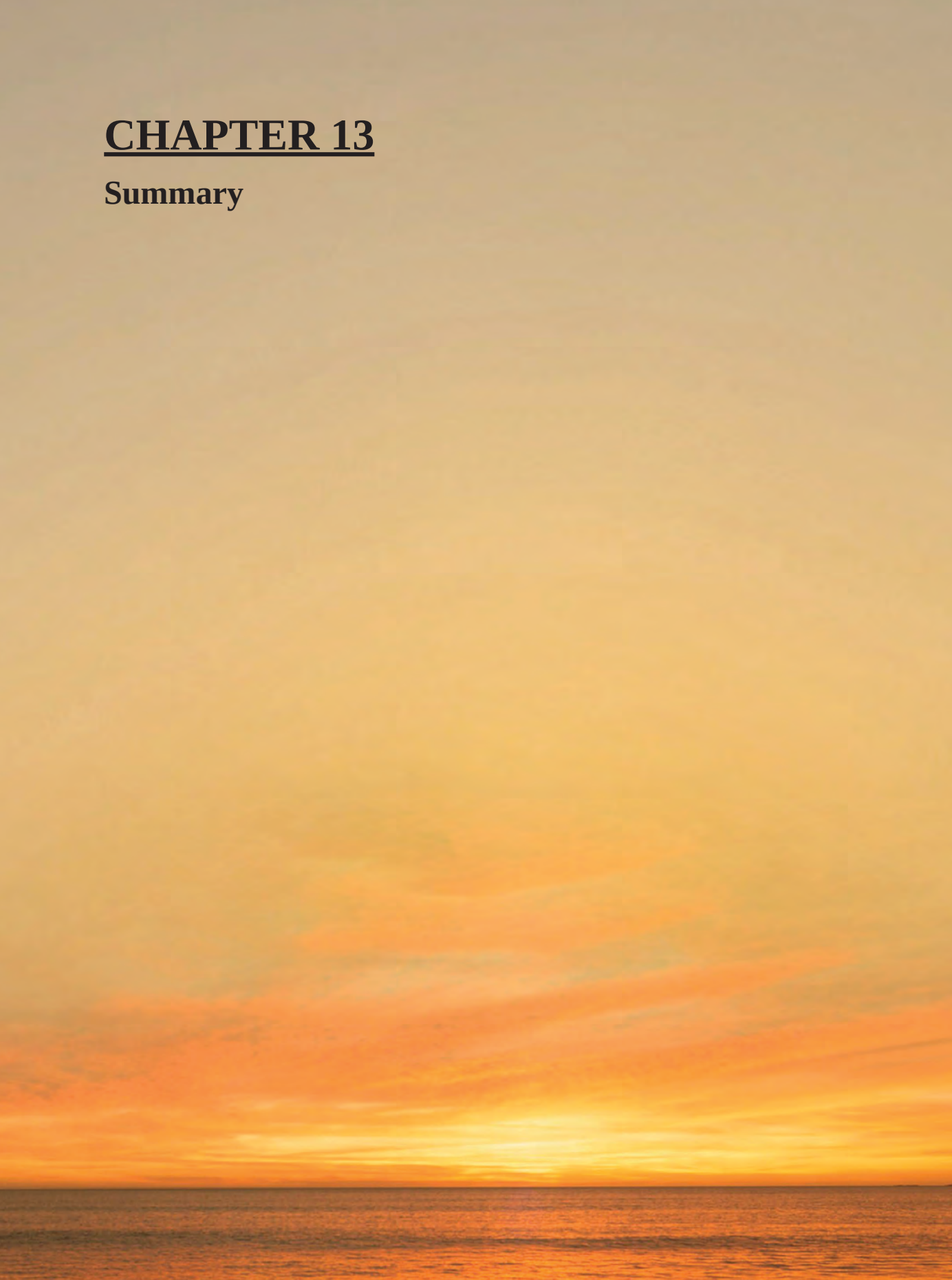
Investigating immune cell trafficking on the ocular surface and its correlation to disease stage and treatment outcomes

CHAPTER 13

Summary



The immunology of the ocular surface has an important role in maintaining ocular homeostasis and in the etiopathogenesis of a number of diseases. As the entire ocular surface is linked via its epithelium, vascular supply and innervation, changes across one aspect has tangible effects on the other. Immune cells resident to and trafficking across the ocular surface interact with the structural tissues to release molecular factors and result in various tissue responses and disease. Corneal and conjunctival epithelial cells and stromal fibroblasts secrete factors like TNF-α, IL-1, IL-6 and IL-8. This interdependence between the cornea structural tissues, inflammation, molecular factors and immune cells has studied in animal models and in-vitro experiments. However, studies evaluating this in-vivo on the human ocular surface are limited and with it a closer representation to actual health and disease in the human eye which is an important lacunae in knowledge . Using various techniques including the ocular surface immune cell wash(OSW), we have tried to demonstrate the immune profile in different diseases and taken this a step forward from existing literature in management of disease. We have found that across different conditions and even within subsets of disease there are distinct immune cells profiles at the ocular surface which interact with the structural cells and the soluble factor milieu in disease. The work included in this PhD, systematically elucidates a broad range of immune cells and molecular factors in disease pathology. Thus helping us find new tools for diseases stratification and monitoring. As the OSW is relatively non-invasive, and repeatable and reliable, it is easily replicable in the out-patient department across different practices.

Many ocular surface diseases have an underlying immune pathogenesis and currently treated predominantly with topical or systemic steroids and systemic immunomodulatory therapy. The innate and adaptive response of the ocular surface is dependent on immune cell trafficking. However, an exaggerated response can result in damage to the cornea and ocular surface and loss of clarity and vision. Some of the diseases in which this response has been found to be implicated include dry eye disease (DED), keratoconus, graft rejection and vascularisation and autoimmune conditions

like Mooren's ulcer, stevens-johnson syndrome and ocular cicatricial pemphigoid. The papers included in this PhD work systemically work towards bridging this knowledge gap and towards clinical trials for evidence based and immune profile based therapies.

Our initial work was on one of the most common immune related ocular diseases we encounter- DED. In the paper on DED we demonstrated that there is a significantly increased proportion of activated neutrophils with macrophage activation in DED patients ($p<0.05$). There was a decrease in the ocular surface NK cells in aqueous deficient eyes with staining and an increase in CD4 and CD8 T cells in DED($p<0.01$). This has important implications for possible targeted therapeutic options in future. As very little of this information was available from actual human ocular surface till now, the knowledge base built in these papers can be instrumental in development of targeted immuno- therapeutic options, especially useful in cases refractory to therapy. For example, activated neutrophils are targeted using antibody therapies. For example, activated neutrophils are targeted using antibody therapies similar to that seen in treatment of certain lung diseases and cancers. In our current practice, autologous serum drops on the local ocular surface immune system by it's immune cell and molecular components.

The importance of understanding the immune cell profile extends to the therapy of these conditions too. We demonstrated the levels of inflammatory markers IL-1b, IL-17F, MMP9, MMP9/TIMP1 ratio and IL-6 were significantly reduced ($p<0.05$) along with improved clinical parameters after intense pulsed light therapy (IPL). There was also a decreasing proportions of NK cells, NKT cells, and T cells, which provides new insights into management of DED. Thus, we are able to understand the effectiveness and applications of newer treatment modalities beyond just MGD using our knowledge of the immune profile. This will help the clinician expand possible therapeutic applications of these treatment modalities as well.

Another common condition that we see in our outpatient department is KC. As it affects a lot of younger population, it is an important disease to find newer treatment and prophylaxis for. Our review articles on keratoconus showed various interleukins

(IL) - IL1a/b, IL-6, IL-8, IL-17A, IL-21, and IL-23 , tumor necrosis factor (TNF), matrix metalloproteinases (MMP) 1, 3, 7, 9 are elevated in tears from KC patients. Lysyl oxidase (LOX) has been found to be decreased in KC and inversely related to severity of the disease. Differential gene expression analysis showed alterations of LOX, collagen I alpha 1 (COLIA1) and collagen IV alpha 1 (COLIVA1) in KC corneal epithelium. The immunophenotyping of KC eyes showed a distinct immune cell profile with a significant increase in the proportions of natural killer (NK) cells and gamma delta T cells in KC. This knowledge provides an opportunity to investigate topical targeted immunomodulation for therapy in KC using Cyclosporine. NK inhibition has rarely been studied. PIBF (progesterone induced blocking factor) is a potential immunomodulator that may find use in KC targeted therapy.

The COVID pandemic brought with it newer challenges of morbidity and mortality. An important problem we faced as health care workers was the mask related ocular surface issues making it an important condition to study. We evaluated ocular surface clinical parameters, tear soluble factors and ocular surface immune cells before and after a prolonged duration of face mask wear. There was a reduction in inflammatory factors IL-6 and IL-8 and increase in other pro-inflammatory factors IL-1, IL-33, TSLP, BDNF and NGF in tears after mask wear. The ocular surface immune cell profile in this cohort was also distinct from DED with a significant increase in the proportion of T cells and a reduction in the proportions of eosinophils, B cells and antibody-producing B cells. In addition, there was a hypo-osmolar change noted. This detailed analysis helps in the better understanding of this condition. Thus immune-biomarker profiling allows us to identify patients accurately and treat them potentially with therapies such a IPL that has shown specific decrease in IL1b and apply appropriate lifestyle changes.

As clinicians it is always important to us to understand the cases that do not fit a pattern or do not respond to treatment as we expect. The next area of work we moved to was patients who had various levels of ocular pain which was not fitting into a diagnosis.

Disparity between signs and symptoms of ocular pain can be a challenge for diagnosis and treatment. Increased dendritic cells (DCs), microneuroma on IVCM and tear molecular factors analysis can help in diagnosis. We formulated an algorithmic approach to diagnosis called the discomfort concordance to signs (DCS) grouping. We found a significant increase in corneal DC density and microneuroma-like structures, along with an altered balance in nociceptive factors, in patients who had pain and ocular discomfort disproportionate to clinical signs, suggesting a neuropathic or nociceptive etiology. This study gives an algorithmic approach to utilizing clinical features, IVCM and molecular factors which can help clinicians diagnose this condition better and provide targeted therapy such as inverse agonists or antagonists of IL17A which are currently in clinical trials for skin inflammation, psoriasis, cancers, etc. [62]

One of the most severe ocular surface diseases we encounter in the clinic is the ocular sequelae of chronic stevens johnson syndrome. This condition is potentially blinding and if we can find the window of opportunity to intervene in the subacute or chronic stage we may be able to save sight. The ocular surface immune profile was studied in chronic SJS patients and showed an increase in activated neutrophils, no change in the NK cell proportions and a significant decrease in the proportions of NK T cells. There was also increased perforin, granzyme and TNFa on the ocular surface which are distinctly different across different phenotypical stages. OSW and tear profile can help clinical management today by stratifying patients at risk of severe sequelae such as keratopathy who require aggressive monitoring and treatment.

We thus demonstrate that the ocular surface immunology has unique patterns and signatures across different diseases. These give us an opportunity to understand the diseases better and find newer targets for therapy and an opportunity to reduce visual morbidity.

Immune cells types on ocular surface	DED	MASK-DE	CLD	MGD (post-Rx)	KC	SJS
Leukocytes	↑	↑	↑	=	=	↑
Neutrophils Total	↑	=	↑	↓	=	↑
Neutrophils (Inactive type)	↑	N.A	↑	N.A	↓	=
Neutrophils (activated type)	↑	N.A	=	N.A	=	↑
Monocytes / Macrophages	↑	=	↑	↓	=	=
NK cells (Total)	=	=	↑	↓	↑	=
NK cells (cytotoxic type)	=	N.A	↑	N.A	↑	=
NK cells (cytokine secreting type)	=	N.A	↑	N.A	↑	=
T cells	↑	=	↑	↓	=	↓
CD4 T cells	↑	N.A	↑	N.A	N.A	N.A
CD8 T cells	↑	N.A	↑	N.A	N.A	N.A
CD4/CD8 ratio	↑	N.A	↑	N.A	N.A	N.A
NK T cells	=	↑	↑	↓	=	↓
Gamma delta T cells	=	↑	=	N.A	↑	=
B cells	N.A	↓	↑	↓	↑	N.A
Neutrophils : T cells ratio	N.A	↓	↑	N.A	↑	↑
Neutrophils : NK cells ratio	↑	=	=	N.A	↓	↑

Table- Our studies across ocular surface conditions have discovered altered cell specific immune responses. The tabular summary of the studies thus far illustrates the unique features of each condition as well as some of the similarities which have been discussed in the text.

CONCLUSION

The immune cell profile has a unique profile in normal versus different ocular surface diseases. The proportions of immune cells resident to and trafficking across the ocular surface is greatly influenced by interactions with local milieu and systemic factors. The change in the immune cell profile can be in response to external or internal stimuli and can be measured by various techniques including impression cytology and OSW. The minimally invasive tests make it easier to monitor the immune profile and has been found to be repeatable across different disease conditions and clinical setups making it a very useful tool in our armamentarium. Across the thesis chapters, we demonstrate the immune cell profile and molecular profile in different conditions. The myriad clinical conditions in which immunology plays a role is known from literature but the understanding of these interactions between immune cells, soluble factors, nerves and corneal epithelial cells directly on the human eye has given us new insight into the molecular pathogenesis of different diseases and definitely reiterated the importance of understanding the ever-changing immune cell milieu in corneal disease. Taken together, the data strongly argues in favour of adopting targeted immunotherapy options from other fields of medicine for topical corneal applications. Clinically, we envisage a move away from steroids and broad action immunomodulators for long term therapy towards biologicals and target specific drugs. This has to be enabled with a broader availability of tear based diagnostics as well as OSW based phenotyping. Such phenotyping tests from blood samples are already mainstay in a variety of haematological conditions, infectious diseases and cancers and therefore their utility is known; we hope to popularise their repurposing for ocular surface practice in the future. In this manner we will bring personalised medicine and patient -centric therapeutic modalities to the ocular surface clinical practice.