

## Monoclonal antibodies in ophthalmology

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### ABSTRACT

The monoclonal antibodies can selectively target specific molecules, proteins or receptors in the body responsible for pathogenesis of a particular disorder. Some cytokines play key role in the development of proliferative diabetic retinopathy, neovascular age related macular degeneration, glaucoma and many other inflammatory conditions of eye. Monoclonal antibodies against VEGF and TNF- $\alpha$  such as bevacizumab, ranibizumab, infliximab and adalimumab have been used to control neovascularization and inflammation in eye with significant positive results whereas others have been used to target CD20, CD52, CD11a, and IL-2. The growing interest in these drugs with current progress in biotechnology and genetic engineering has kindled active research and with more understanding of the molecular basis of many ocular disorders these antibodies are being explored in a variety of different pathological conditions of the eye. Various sight threatening serious eye disorders which are resistant to the conventional available therapy have responded favorably to these drugs. Despite the limitations of high cost and uncertainty around long term safety profile, rational use of the monoclonal antibodies holds immense promise in management of various ocular conditions.

### INTRODUCTION

Various growth factors and cytokines are involved in cellular and vascular proliferations in the eye. Some of these are also involved in the pathogenesis of different ophthalmologic diseases. One way to regulate these molecules or their receptors is to target them through specific antibodies. Older ways of producing antibodies for targeting such specific endogenous molecules had certain limitations. Normally the immune system produces different types of antibodies with varying affinity in response to a single antigen. It was necessary to narrow this natural polyclonal antibody producing response to antibody production of one type with single specificity. The production of the antibodies by earlier methods also gave limited yields as the tissues in which they were grown had a particular fixed lifespan. The introduction of monoclonal antibody (mAb) - the required antibody of a single class with particular specificity and affinity, which could be easily and continuously produced- paved the way to overcome these limitations. In 1975, Kohler and Milestone made the first monoclonal antibody by fusing the specific antibody producing spleen cell with a continuously dividing myeloma cell.<sup>1</sup>

### PRODUCTION OF MONOCLONAL ANTIBODIES<sup>2-4</sup>

The initial step in monoclonal antibody production involves the fusion of immortal myeloma cells with the

specific antibody producing spleen cells. The resulting fused cells or hybridomas are selected by their ability to grow in a medium made hostile for other non-hybrid cells. The selected hybridomas are then screened for particular antigenic specificity and further used for producing monoclonal antibodies.

*Hybridoma production:* Successful fusion of one somatic cell with the other, producing hybrid cell or heterokaryon, was done in the early 1970s. The fusion of membranes of the two cells was facilitated by agents such as inactivated enveloped virus or polyethylene glycol. Thus initially an unstable single hybrid cell was produced with cytoplasm and nuclei of more than one cell, which later was transformed into a stable fused cell with a single large nucleus. But all cells do not fuse and even in the population of fused cells most are the cells which are the fusion of two cells of a single parent type (A-A or B-B) instead of being the hybrid product (A-B) of two different cell types. The needed smaller population of hybrid cells has to be selected out of the unfused or non-hybrid fused cells. HAT medium (consisting of hypoxanthine, aminopterin, and thymidine) helps in selecting the hybrid cells out of this heterogenous mixture of cells.

The denovo pathway of nucleotide synthesis in mammalian cells can be blocked by aminopterin, a folic acid analog, present in the HAT medium. But the cell can still survive by synthesizing nucleotides using

salvage pathway which involves the enzymes hypoxanthine-guanine phosphoribosyl transferase (HGPRT) and thymidine kinase (TK). Mutation in one of these enzymes can block this alternate salvage pathway necessary for the cell survival.

The myeloma cells used for the purpose of monoclonal antibody production are HGPRT<sup>-</sup> so cannot grow by using the salvage pathway in HAT medium. Spleen cells (which have limited growth capacity) primed with particular antigen are fused with the HGPRT<sup>-</sup> myeloma cells. Only hybridoma clusters survive the HAT medium after 7-10 days. Single cells from such clusters are propagated to maintain the monoclonal nature of the antibody.

*Screening of hybridomas:* Not all hybridomas finally produced, secrete the antibodies specific for the target antigen. Some of the hybridomas may produce antibodies specific to unwanted antigens. For screening desired antibody producing hybridomas usually ELISA and radioimmunoassay techniques are applied.

*Antibody production:* Once the hybridomas producing antibodies of the particular antigen specificity are screened they are cloned again with restricted dilution to get the monoclonal culture. Then these are grown in the tissue culture flask to produce the desired monoclonal antibody. In the tissue culture medium monoclonal antibodies are produced in low concentrations (10-100 µg/ml). Production rate is higher in the ascitic fluid medium of histocompatible mice, from which the monoclonal antibodies are separated later by chromatography. Several other techniques have been evolved for the higher rate of production of the monoclonal antibodies.

Initial mAbs were of murine origin but they were less efficacious when used in human beings. Their use also produced unwanted immunogenic response. The use of genetic engineering with advanced biotechnology techniques further helped to overcome these problems by progressively increasing the substitution of the murine sequences with the human parts of the antibody. This made possible the development of the mixed human-murine (chimeric) mAb, and later the fully human monoclonal antibody. The generic name of a monoclonal antibody ends with "(-mab)". The name is further specified by "(-ximab)" if it is chimeric (mixed) and "(-umab)" if it is human monoclonal antibody. Currently more than twenty mAbs are available for use in various cancers and inflammatory conditions and hundreds are under development.

### RELEVANCE IN OPHTHALMOLOGY:

The development of therapeutic monoclonal antibodies (mAbs) provided many great advances in the treatment

of some diseases, especially cancers. The molecular pathways, initiated by vascular endothelial growth factor (VEGF), platelet-derived growth factor, integrins, etc are the current topics of interest in developing anti-angiogenesis treatment options. The development of mAbs has specially made a paradigm change in the management of tumors by targeting the tumor angiogenesis. They are also being increasingly used in other fields of medical science, including ophthalmology and have become an important alternative therapeutic choice in some serious ocular conditions. Some of these mAbs such as ranibizumab, bevacizumab, infliximab, and adalimumab can control neovascularization and inflammation. They have found very effective application in ophthalmology, especially in angiogenic and inflammatory conditions. Rituximab, daclizumab, efalizumab, and alemtuzumab have also shown to be effective as adjunctive therapy in various ocular problems in many preclinical and early clinical studies.<sup>5</sup>

Angiogenesis plays an important role in the pathogenesis of many ocular disorders including neovascular age-related macular degeneration (AMD), proliferative diabetic retinopathy and glaucoma. Inflammation, hypoxia and ischemia are some of the proangiogenic factors, which induce the formation of new blood vessels. VEGF, a key regulator of the angiogenesis pathway, is increased during these states. It selectively brings about significant changes in the endothelial cells which interact with pericytes and the matrix molecules leading to cellular proliferation and migration. It is also a very potent mediator of vascular permeability. Preclinical and clinical research has established the important role of VEGF in the pathogenesis of choroidal neovascularization (CNV). It is the main mediator of proliferative diabetic retinopathy and macular edema. Monoclonal antibodies blocking VEGF have revolutionized the management of proliferative retinopathy and neovascular AMD. When given as intravitreal injections they decrease the proliferation and leakage from choroidal neovascular lesions and help in arresting the disease process and improving vision.<sup>6</sup> Ranibizumab and bevacizumab are the two main VEGF inhibitor mAbs currently being used in various ocular disorders.

Tumor necrosis factor-alpha (TNF- $\alpha$ ) and interleukin 1 (IL-1) are important inflammatory mediators. TNF- $\alpha$  is a cytokine produced by macrophages and many other cells. It plays a vital role in endogenous immune reaction and inflammation. It also induces the expression of other proinflammatory cytokines and adhesion molecules. It has been implicated in various inflammatory conditions including autoimmune uveitis. In experimental autoimmune uveoretinitis, TNF- $\alpha$  is found to play crucial

role in inflammation and tissue damage. Levels of TNF- $\alpha$  have been found to be raised in ocular fluid and serum of patients with uveitis.<sup>7</sup> Serious cases of pan-or-posterior uveitis have conventionally been treated with systemic corticosteroids and other immunosuppressive drugs such as cyclosporine, tacrolimus, methotrexate, mycophenolate mofetil, azathioprine etc. Patients who are unresponsive to these interventions can be managed with TNF-alpha inhibitors, such as infliximab and adalimumab.

CD20, CD52, CD11a, and IL-2 are some of the other molecules which are significantly involved in the pathogenesis of many ocular disorders and many mAbs are being tried to target these for effective therapy.

### **BEVACIZUMAB**

Bevacizumab is a recombinant humanized monoclonal antibody which inhibits the binding of VEGF to its receptors, Flt-1 and KDR, on the surface of endothelial cells. It is safe and effective in patients with proliferative diabetic retinopathy who do not respond favorably to pan retinal photocoagulation.<sup>8</sup> It can be used as an adjunct to vitrectomy in the management of severe proliferative diabetic retinopathy (PDR). An intravitreal injection of bevacizumab seven days preoperatively has been suggested as a new strategy for the surgical treatment of severe PDR because this can help in smoother and safer surgery by reducing retinal and iris neovascularization, thus providing better anatomical and functional outcome.<sup>9</sup> Intravitreal injection of bevacizumab can effectively treat diabetic papillopathy. After diabetic vitrectomy it can reduce the need of repeat vitrectomy indicated for recurrent vitreous hemorrhage.<sup>10,11</sup>

Myopic maculopathy is one of the leading causes of subfoveal choroidal neovascularization (CNV) among elderly patients. Intravitreal bevacizumab can be effective for subfoveal and juxtafoveal CNV in highly myopic eyes but the outcome may not be sustained for long term and becomes insignificant by the end of the second year. In exudative ARMD multiple injections of bevacizumab produced improvement in visual acuity but the improvement could not be sustained after two years.<sup>12,13</sup>

Bevacizumab has been used successfully for macular edema and intravitreal bevacizumab is as effective as triamcinolone acetonide for refractory uveitic cystoid macular edema.<sup>14</sup> Resolution of macular edema in a case of Coats' disease has also been reported with intravitreal bevacizumab.<sup>15</sup>

It can be used as adjunctive therapy in advanced retinopathy of prematurity (ROP) to facilitate better outcome along with the standard laser photocoagulation and surgery especially in cases with incomplete

regression with laser alone. The combination of laser photocoagulation and intravitreal bevacizumab injection seems to be well tolerated, induces fast regression of aggressive zone-1 retinopathy of prematurity without retinal detachment and is well tolerated.<sup>16-18</sup> More studies will be needed to establish its role as the first line therapy.

Short term improvement in visual acuity with neovascular AMD can be achieved by three consecutive monthly injections of intravitreal bevacizumab.<sup>19</sup> Longer prospective studies are needed to see the efficacy in sustaining this positive result.

Bevacizumab has also been used in select patients with idiopathic persistent central serous chorioretinopathy with encouraging results.<sup>20</sup>

Monoclonal antibodies targeting the VEGF have been giving promising results in the management of neovascular glaucoma and wound modulation after trabeculectomy surgery. Their use has resulted in regression of the neovascularisation of iris and anterior chamber angle and helped in the survival of blebs. There is better control of intraocular pressure and improvement in symptoms. Topical bevacizumab has been successfully used in cases of early bleb failure.<sup>21, 22, 23</sup> Interestingly when used for other ocular problems with no previous diagnosis of glaucoma or ocular hypertension multiple intravitreal anti-VEGF injections have led to sustained increase in IOP sometimes requiring IOP lowering therapy.<sup>24</sup>

One study for the management of retinal angiomatous proliferation suggests that bevacizumab if given along with photodynamic therapy produces synergistic effect and the required rate of the intravitreal re-injections may decrease.<sup>25</sup>

Increased VEGF, with inflammation and fibro vascular proliferation, plays an important role in the development of the pterygium. The over expression of VEGF in pterygium tissue and the growth of blood vessels can be treated with subconjunctival bevacizumab. Blockade of VEGF after a single subconjunctival injection of ranibizumab or bevacizumab caused prompt regression of conjunctival microvessels in the eyes with inflamed pterygium or residual pterygia.<sup>26</sup> But it was not found to be effective in preventing recurrence of pterygia. In a study, when bevacizumab was given as single intraoperative subconjunctival injection to evaluate its efficacy as an adjunctive therapy for primary pterygium surgery, there were no statistically significant differences regarding reduction in recurrence rate or early postoperative conjunctival erythema, lacrimation, refractive astigmatism, improvement in visual acuity, corneal epithelial defects, or photophobia between the

case and control groups.<sup>27</sup> One study though showed bevacizumab to delay the recurrence by inhibiting the growth in impending recurrent pterygium.<sup>28</sup>

Corneal neovascularization is a very important risk factor for graft failure after corneal transplantation. Bevacizumab has been used effectively for improvement of prognosis in high-risk corneal transplantation with pre- and postoperative corneal neovascularization. Subconjunctival, perilimbal, and intrastromal injection of bevacizumab have been well tolerated without any significant side effect.<sup>29</sup>

### **RANIBIZUMAB**

Ranibizumab is a recombinant, fully humanized monoclonal anti-VEGF-A antibody. It prevents the interaction of VEGF with the endothelial cells thus inhibiting cellular proliferation, new vessel formation and vascular leakage. Intravitreal anti-angiogenic treatment with ranibizumab can significantly decrease the intraocular VEGF expression below physiologic levels compared with controls. This effect persists for as long as 4 weeks after each injection and is prolonged by consecutive retreatment.<sup>30</sup>

Intravitreal ranibizumab is effective in CNV associated with idiopathic central serous chorioretinopathy. However, residual intraretinal or sub retinal fluid seems to persist along with the increased choroidal permeability. It has been found to be effective in CNV associated with punctate inner choroidopathy and improvement in vision was observed. On recurrence of choroidal neovascularisation in such case additional ranibizumab injections can be safely and successfully repeated.<sup>31,32</sup> A case of CNV in association with retinal astrocytic hamartoma was seen to respond favorably to intravitreal ranibizumab.<sup>33</sup> It has shown positive results as monotherapy in the management of retinal angiomatous proliferation in age related macular degeneration. Intravitreal ranibizumab reduces the risk of loss of vision and increases the chance of improving visual acuity compared with no treatment or photodynamic therapy for selected cases of subfoveal choroidal neovascularization (CNV) in age-related macular degeneration (AMD).<sup>34</sup> In myopic choroidal neovascularization, intravitreal ranibizumab produces improvement in visual acuity and reduces retinal thickness.<sup>35</sup>

It has also been shown to be effective in idiopathic retinal vasculitis, aneurysms, neuroretinitis, and Coats' disease associated with muscular dystrophy.<sup>36,37</sup> Intravitreal ranibizumab can be used safely in the treatment of cystoid macular edema associated with retinitis pigmentosa.<sup>38</sup>

### **INFLIXIMAB**

Infliximab is a chimeric anti-TNF-alpha monoclonal antibody containing a murine TNF-alpha binding region and human IgG1 base. It has been tried successfully in various pathological conditions of eye.

It has been effectively used for managing the refractory uveoretinitis in Behçet's disease. There is significant reduction in inflammation, improvement of visual acuity, and decrease in ocular complications and relapse with infliximab therapy as compared to conventional therapy.<sup>39</sup> Non-infectious scleritis, a serious inflammatory condition, can respond to infliximab when the conventional therapy with systemic steroids is not effective. A favorable clinical response can be achieved in three months and the remission can be maintained with repeat monthly infusions.<sup>40</sup> In severe ocular rheumatoid disease, episcleritis can progress to the stage of scleromalacia perforans. Infliximab accompanied by methotrexate and prednisone can produce dramatic remission in such condition.<sup>41</sup>

TNF-alpha is also implicated as one of the factors important for neovascularization. In preclinical studies infliximab was shown to reduce both the size and the leakage of laser-induced choroidal neovascularisation.<sup>42</sup> Intravitreal infliximab can help in control of neovascular age-related macular degeneration though sufficient studies have not yet been conducted for this indication.<sup>43</sup>

Infliximab is effective in treating Vogt-Koyanagi-Harada disease, and can be the steroid sparing agent.<sup>44</sup> It can also be effective in treatment of retinal vascular tumors. When given in a case of macular cavernous hemangioma there was marked involution of the tumor and improvement in visual acuity.<sup>45</sup>

Other ocular conditions where infliximab has been effectively used are refractory retinal vasculitis due to sarcoidosis, diffuse subretinal fibrosis syndrome, idiopathic sclerosing orbital inflammation (myositis) when given along with methotrexate and sight threatening thyroid associated ophthalmopathy.<sup>46,47,48,49</sup>

### **ADALIMUMAB**

Adalimumab is a fully human IgG1 monoclonal antibody that specifically binds to tumor necrosis factor (TNF)-alpha. Apart from directly inhibiting TNF-alpha activity it also induces the apoptosis of TNF-expressing mononuclear cells. It is given subcutaneously.

Adalimumab seems to be an effective and safe therapy for the management of sight threatening refractory uveitis and may help to reduce the use of concomitant immunosuppressive drugs.<sup>50</sup> It is effective in juvenile idiopathic arthritis-associated chronic anterior uveitis

and psoriatic ocular inflammation. It can be an effective alternative in treating severe nodular scleritis - a common ocular manifestation of rheumatoid arthritis - especially in patients who do not respond to conventional treatment of steroids and DMARDs.<sup>51, 52, 53</sup>

Adalimumab can also be a good alternative in those patients also who are hypersensitive to infliximab. Even without hypersensitivity if the initial ocular response to one antibody starts diminishing after sometime, the clinical recovery can be maintained by switching to another antibody. This kind of switching between the antibodies may also help to control systemic symptoms and allow easy administration.<sup>54, 55</sup>

### **RITUXIMAB**

Rituximab is a chimeric anti-CD20 monoclonal antibody. It has a unique mode of action and can induce the inhibition of CD20+ cells via multiple mechanisms. It can act directly by complement-mediated and antibody-dependent cell-mediated cytotoxicity, and indirectly by bringing about structural changes, apoptosis, and sensitization of CD20 cells to chemotherapy.<sup>56</sup> Rituximab has been shown to be effective in ocular adnexal lymphoma as most of these are low grade, B cell, non-Hodgkin's lymphoma. Benign lymphoid hyperplasia of the orbit and vitreoretinal lymphoma have responded favorably to this drug.<sup>57,58</sup> It has shown to cause regression in untreated mucosal-associated lymphoid tissue (MALT) lymphoma but no positive result was observed in relapsing patients.<sup>59</sup> One study describes the complete response in a patient of a primary conjunctiva lymphoma with conventional dose of rituximab without appreciable toxicity.<sup>60</sup>

Remission has been achieved in refractory bilateral retinal vasculitis, a dangerous complication of systemic lupus erythematosus (SLE) through rituximab-induced B-cell depletion.<sup>61</sup> Rituximab has also been effectively used to produce rapid improvement in symptoms of progressive, corticosteroid-resistant thyroid-associated ophthalmopathy with effective resolution of orbital inflammation and dysthyroid optic neuropathy.<sup>62</sup> Clinical improvement has been seen with rituximab therapy in refractory ophthalmic Wegener's granulomatosis.<sup>63</sup> Rituximab is effective in reversing papilledema and cerebral sinus thrombosis, and preserving the vision in antiphospholipid antibody syndrome patients resistant to treatment with systemic steroids and immunoglobulin. The safety profile and compliance is also found to be better.<sup>64</sup> It produces positive improvement in Sjögren syndrome as B cells are also important in pathogenesis of this disorder. Severe keratoconjunctivitis sicca refractive to conventional treatment can respond favorably upon treatment with rituximab.<sup>65</sup> It has also

been found to be effective in recalcitrant ocular cicatricial pemphigoid when given along with intravenous immunoglobulin.<sup>66</sup>

### **OTHER ANTIBODIES**

**Daclizumab** is a humanized IgG1 monoclonal antibody which binds with the alpha subunit of the interleukin-2 receptor. It can effectively control recalcitrant ocular inflammation and can be used long term in children and adults as corticosteroid sparing agent to induce sustained remission.<sup>67</sup> High-dose intravenous daclizumab has been shown to be effective in the treatment of juvenile idiopathic arthritis-associated active anterior uveitis.<sup>68</sup> Daclizumab has been found to be a safe and effective therapy in most patients with birdshot retinopathy, resulting in vision stabilization and resolution of inflammation.<sup>69</sup> It has also been found to be effective in decreasing the intraocular inflammation in Vogt-Koyanagi-Harada's disease, bilateral idiopathic panuveitis and bilateral idiopathic intermediate uveitis.<sup>70</sup>

A few other mAbs which have been tried for ophthalmic conditions are **efalizumab**, **alemtuzumab**, and **basiliximab**. Efalizumab acts by binding to CD11a, the alpha-subunit of leukocyte function antigen-1 (LFA-1). By inhibiting the binding of LFA-1 to ICAM-1, efalizumab interferes with the adhesion of leukocytes to other cell types and the migration of T lymphocytes to sites of inflammation.<sup>71</sup> Basiliximab is a chimeric monoclonal interleukin 2-receptor antibody and inhibits T-cell proliferation. Alemtuzumab targets CD52, a protein present on the surface of mature lymphocytes.

There is not enough literature on the use of these drugs in ophthalmology but these drugs have been explored in a few ocular disorders. Efalizumab has been tried in resistant uveitis. Alemtuzumab has produced positive response and sustained useful vision during follow-up in cancer-associated retinopathy and optic neuropathy.<sup>72</sup> Basiliximab, when compared in a study with cyclosporine for safety and efficacy after penetrating risk keratoplasty, showed a lower efficacy in preventing immune reactions though the side effect profile was better.<sup>73</sup>

### **CONCERNS**

One of the biggest limitations in the use of mAbs is their high cost, and this factor becomes more relevant for the developing countries where majority of the common people can't afford these. The safety concerns regarding the ocular use of mAbs pertains to the inherent risks of the intravitreal route of administration, ocular toxicities of the particular mAb, and the systemic side effects. The risks with intravitreal injection procedure for mAb administration are as for any other drug. As far as local

toxicity of the drug is concerned different types of adverse drug reactions have been reported as case reports in the medical literature such as severe ocular inflammation, retro bulbar optic neuritis, non-arteritic anterior ischemic optic neuropathy, corneal melt, retinal toxicity, isolated sixth nerve palsy, etc.<sup>74,75,76,77</sup> But these case reports have been infrequent and overall the ocular use of the mAbs is without significant adverse reactions and relatively safe.

There can be a development of immunogenic reaction to the antibody. The mixed monoclonal antibodies have a greater propensity to induce immunogenicity and cause allergic reactions than the humanized ones.

After intravitreal administration, the mAbs have been detected in the systemic circulation so there is a potential of systemic adverse reactions with their use despite most of the randomized clinical trials showing them to be safe and devoid of systemic reactions.<sup>78</sup> Most of the clinical trials enroll a number of patients which is much less than that required to detect the incidence of any uncommon adverse reaction. Some unexpected chronic adverse effects can also be noticed after these drugs are around for a while, because the restricted period of exposure in the clinical trial phases for safety studies cannot predict all the adverse reactions. So a general alert during the post marketing period should be there when these drugs are actually being used in a large population base increasing the chances of incidence of potential adverse effects. Pharmacovigilance for these drugs during the initial years can further reassure of their safety during long term use.

## CONCLUSION

The advent of mAbs in ocular therapeutics has increased our alternatives in the armamentarium against some sight threatening serious conditions of the eye which are resistant to the normal conventional treatments available. With more molecular pathways being defined and the drug development process getting revolutionized by genetic engineering and biotechnology, newer important receptors will be targeted in future by the monoclonal antibodies. We can hope to see the emergence of safer, cheaper and easily administered monoclonal antibodies, as better alternatives for various pathological conditions of the eye.

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