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(54) **Title:** METHOD OF TREATMENT OF AGE-RELATED MACULAR DEGENERATION

(57) **Abstract:** The invention provides methods and compositions for treatment of age-related macular degeneration, which comprises causing T cells that produce IL-4 to accumulate in the eye by administration of an agent such as Copolymer-1, IL-4, cells activated with IL-4, IL-13 or up to 20 ng/ml IFN-g, or a pathogenic self-antigen associated with a T-cell-mediated specific autoimmune disease of the eye or a peptide derived therefrom, and any combination of such agents.

METHOD OF TREATMENT OF AGE-RELATED MACULAR DEGENERATION

FIELD OF THE INVENTION

The present invention relates to methods and compositions for treatment of age-related macular degeneration.

Abbreviations: **Aβ**, amyloid β-peptide; **AD**, Alzheimer's disease; **AMD**, age-related macular degeneration; **APCs**, antigen-presenting cells; **BrdU**, 5-bromo-2'-deoxyuridine; **CNS**, central nervous system; **COP-1**, copolymer 1; **GFP**, green fluorescent protein; **IB-4**, *Bandeiraea simplicifolia* isolectin B4; **IGF**, insulin-like growth factor; **IL**; interleukin; **MG**, microglia; **MHC-II**, class II major histocompatibility complex; **MWM**, Morris water maze; **NPCs**, neural stem/progenitor cells; **RGCs**, retinal ganglion cells; **Tg**, transgenic; **Th**, T-helper; **TNF-α**, tumor necrosis factor-α.

BACKGROUND OF THE INVENTION

Age-related macular degeneration

Age-related macular degeneration (AMD) is a disease affecting the macular region of the eye, which is the area in the retina where the sharp vision is obtained. Macular degeneration is caused by the deterioration of the central portion of the retina, the inside back layer of the eye that records the images we see and sends them via the optic nerve from the eye to the brain. The retina's central portion, known as the macula, is responsible for focusing central vision in the eye, and it controls our ability to read, drive a car, recognize faces or colors, and see objects in fine detail.

AMD is the leading cause of irreversible blindness among the elderly in industrialized nations, and its prevalence increases in the population over the age of 60 (Klein et al., 1992; Mitchell et al., 1995). Numerous attempts have been made to understand the etiology of the disease, its pathophysiology and factors involved in the progression of the disease. A common early sign of AMD is the buildup of drusen, tiny yellow or white fat globules and extracellular material in the retina of the eye or on the optic nerve head. Drusen occurs as hard drusen (small, solid deposits that seem harmless) or larger deposits of soft drusen with indistinct borders. Soft drusen accumulating between the retinal pigment epithelium (RPE) and Bruch's membrane force these two structures apart.

Most people over 40 have a small amount of hard drusen, which can join to form soft drusen in AMD cases. However, not all soft drusen come from hard drusen.

There are two types of macular degeneration: the dry or atrophic type, and the wet or hemorrhagic type. The dry form of AMD, which constitutes 80% of all AMD patients, is characterized by the appearance of drusen. The presence of drusen is considered to be a pre-existing factor associated with the progression of the disease to either advanced dry AMD or wet AMD.

Alzheimer's disease

Alzheimer's disease (AD) is an age-related progressive neurodegenerative disorder characterized by memory loss and severe cognitive decline (Hardy & Selkoe, 2002). The clinical features are manifested morphologically by excessive accumulation of extracellular aggregations of amyloid β-peptide (Aβ) in the form of amyloid plaques in the brain parenchyma, particularly in the hippocampus and cerebral cortex, leading to neuronal loss (Selkoe, 1991). In addition, in most mouse models of Alzheimer's disease the neurogenesis that normally occurs throughout life in the hippocampus of the adult brain is disrupted (Haughey et al., 2002). In Alzheimer patients, like in transgenic mice (PDGF-APPSw, Ind), some increase in neurogenesis takes place but is apparently not sufficient to overcome the disease (Jin et al., 2004a, b).

The similarity between AMD and Alzheimer's disease

AMD and Alzheimer's disease are both chronic neurodegenerative disorders that affect a substantial proportion of elderly persons. Characteristic of these disorders is the irreversible loss of function, for which there is no cure. The degeneration occurring in AMD and Alzheimer's disease may, to some extent, have a common pathogenesis (Klaver et al., 1999). Although the etiology of both AMD and Alzheimer's disease is largely unknown, the pathogeneses of the two diseases show some striking similarities. In AMD, early histopathological manifestations are extracellular drusen deposits and basal laminar deposits (Hageman & Mullins, 1999). These lesions contain lipids, glycoproteins and glycosaminoglycans, which are presumably derived from a degenerating neuroretina (Kliffen et al., 1995). Accumulation of these deposits is associated with loss of photoreceptors and subsequent deterioration of macular function (Holz et al., 1994). As noted above, an early pathologic hallmark in Alzheimer's disease is the presence of extracellular senile plaques (Selkoe, 1991). These plaques are composed of many components, including small peptides generated by proteolytic cleavage of a family of transmembrane polypeptides known as amyloid precursor proteins. Two peptides that are widely regarded as major contributors to the pathology of Alzheimer's disease are known as amyloid-β (Aβ) peptides. Shared components of amyloid deposits and drusen include proteins such as vitronectin, amyloid P, apolipoprotein E, and even the Aβ peptides and amyloid oligomers that are associated with amyloid plaques in Alzheimer's disease (Luibl et al., 2006; Mullins et al., 2000; Yoshida et al., 2005).

The Aβ peptides present in Alzheimer's disease activate microglial cells to produce potentially neurotoxic substances such as reactive oxygen and nitrogen species, proinflammatory cytokines, complement proteins, and other inflammatory mediators that bring about neurodegenerative changes (Akiyama et al., 2000). The inflammatory response that has been associated with Alzheimer's disease often involves $CD11b^+$ activated microglia, representing the innate arm of the immune system in the central nervous system (CNS) (Streit, 2004). $CD11b^+$ microglia were reported to be associated with age-related normal human brain (Streit, 2004), and it is possible that such microglia are the ones that contribute both to age-related cognitive loss and to impaired neurogenesis (Monje et al., 2003). CD11b have also been found in patients with Alzheimer's disease (Akiyama & McGeer, 1990). Moreover, inflammatory mediators are present in amyloid deposits as well as in drusen, suggesting a possible common role for the inflammatory pathway in AMD and Alzheimer's disease (Hageman et al., 2001). A role for local inflammation in drusen biogenesis suggests that it is analogous to the process that occurs in Alzheimer's disease, where accumulation of extracellular plaques and deposits elicits a local chronic inflammatory response that exacerbates the effects of primary pathogenic stimuli (Akiyama et al., 2000).

Microglial activation in neurodegeneration

Microglia are bone marrow-derived glial cells, which are present within all layers of the adult human retina (Penfold et al., 1991). Several types are present which may be associated with neurons or with blood vessels, and some of these are antigen-presenting cells (APCs) (Penfold et al., 1991; Provis, 2001). The nature of microglial activation, either beneficial or harmful, in damaged neural tissue depends on how microglia interpret the threat (Butovsky et al., 2005). Although the presence of microglial cells in normal undamaged neural tissue has been debated for years, it is now an accepted fact (Nimmerjahn et al., 2005), including their presence in the eye. The role of microglia in inflammatory processes is controversial. On the one hand, participation of microglia in inflammatory process of the eye can stimulate mature retinal ganglion cells (RGCs) to regenerate their axons (Yin et al., 2003). On the other hand, the role of microglia in neurodegenerative processes may be detrimental to the neuronal tissue. Roque et al (1999) showed that microglial cells release soluble product(s) that induce degeneration of cultured photoreceptor cells. This controversy may be explained by the contradicting reports regarding the presence of antigen-presenting cells, which are crucial factors of an antigen-specific cell-mediated immune response. Immunological responses in neural retinal microglia are related to early pathogenic changes in retinal pigment epithelium pigmentation and drusen formation. Activated microglia may also be involved in rod cell death in AMD and late-onset retinal degeneration. A recent study has proposed that microglia, activated by primary rod cell death, migrate to the outer nuclear layer, remove rod cell debris and may kill adjacent cone photoreceptors (Gupta et al., 2003).

Like blood-derived macrophages, microglia exhibit scavenging of extracellular deposits, and phagocytosis of abnormal amyloid deposits in Alzheimer's disease. Such microglia, while efficiently acting as phagocytic cells, cause neuronal death by the secretion of mediators like tumor necrosis factor alpha (TNF-α) (Butovsky et al., 2005), and thus, while acting as phagocytic cells (Frenkel et al., 2005), they are apparently not efficient enough to fight off the Alzheimer's disease symptoms. In contrast to these resident microglia, microglia derived from the bone marrow of matched wild-type mice can effectively remove plaques (Simard et al., 2006). Moreover, an absence of normally functioning macrophages lead to the development of clinical AMD (Ambati et al., 2003). Thus, AMD, like Alzheimer's disease, illustrates a disease in which scavenging of abnormal deposits inevitably induces self-perpetuation of disease progression mediated by the phagocytic cell themselves (Gupta et al., 2003).

Protective autoimmunity

Some years ago our group formulated the concept of 'protective autoimmunity' (Moalem et al., 1999). Both pro-inflammatory and anti-inflammatory cytokines were found to be critical components of a T cell-mediated beneficial autoimmune response, provided that the timing and the intensity of the T-cell activity was suitably controlled (Butovsky et al., 2005; Shaked et al., 2004), and depending on the nature of the disease (Schwartz et al., 2006). According to our concept, an uncontrolled autoimmunity leads to the commonly known condition of autoimmune diseases associated with overwhelmed activation of microglia (Butovsky et al., 2006a), as will be discussed below. The beneficial effect of the autoreactive T cells was found to be exerted via their ability to induce the CNS-resident microglia to adopt a phenotype capable of presenting antigens (Butovsky et al., 2001; Butovsky et al., 2005; Schwartz et al., 2006; Butovsky et al., 2006a; Shaked et al., 2004), expressing growth factors (Butovsky et al., 2005; Butovsky et al., 2006a;b), and buffering glutamate (Shaked et al., 2005).

In attempting to boost the efficacy of the protective autoreactive T cells, we tested many compounds in the search for a safe and suitable antigen for neuroprotection. We then suggested to use glatiramer acetate, also known as Copolymer 1 or Cop-1 (Kipnis et al., 2000; Avidan et al., 2004; Angelov et al., 2003), a synthetic 4-amino-acid copolymer known to be safe and currently used as a treatment for multiple sclerosis by a daily administration regimen (Copaxone®, Teva Pharmaceutical Industries Ltd., Israel). In our studies we have demonstrated its low-affinity cross-reaction with a wide range of CNS autoantigens. Because the affinity of cross-reaction is low, the Cop-1-activated T cells, after infiltrating the CNS, have the potential to become locally activated with little or no attendant risk of autoimmune disease (Kipnis et al., 2000).

A single injection of Cop-1 is protective in acute models of CNS insults (Kipnis et al., 2000; Avidan et al., 2004; Kipnis. & Schwartz, 2002), while in chronic models occasional boosting is required for a long-lasting protective effect (Angelov et al., 2003). In the rat model of chronically high intraocular pressure, vaccination with Cop-1 significantly reduces RGC loss even if the pressure remains high. It should be noted that the vaccination does not prevent disease onset, but can slow down its progression by controlling the local extracellular environment of the nerve and retina, making it less hostile to neuronal survival and allowing the RGCs to be better able to withstand the stress (Schori et al., 2001; Benner et al., 2004; Kipnis & Schwartz, 2002; Kipnis et al., 2000).

For chronic conditions occasional boosting is needed. For example, in a model of chronically elevated intraocular pressure, weekly administration of adjuvant-free Cop-1 was found to result in neuroprotection (Bakalash et al., 2005). The neuroprotective effect of Cop-1 has been attributed in part to production of brain-derived neurotrophic factor (BDNF) (Kipnis et al., 2004b; Ziemssen et al., 2002).

Aggregated Aβ induces toxicity on resident microglia and impairs cell renewal

Recent studies performed in our laboratory suggested that microglia exposed to aggregated Aβ, although effective in removing plaques, are toxic to neurons and impair neural cell renewal (Butovsky et al., 2006a); these effects are reminiscent of the response of microglia to invading microorganisms (as exemplified by their response to LPS) (Butovsky et al., 2005; Schwartz et al., 2006). Such activities are manifested by increased production of TNF-α, down-regulation of insulin-like growth factor (IGF-I), inhibition of the ability to express class II major histocompatibility complex (MHC-II) proteins and CD11c (a marker of dendritic cells) and thus to act as antigen-presenting cells (APCs), and failure to support neural tissue survival and renewal ((Butovsky et al., 2006a; Butovsky et al., 2005; Butovsky et al., 2006b). Further, we found that when microglia encounter aggregated β-amyloid, their ability to remove these aggregates without exerting toxic effects on neighboring neurons or impairing neurogenesis depends upon their undergoing a phenotype switch. A switch in microglial phenotype might take place via a local dialog between microglia and T-cells, which is mediated by T cell-derived cytokines such as interleukin (IL)-4. Addition of IL-4, a cytokine derived from T-helper (Th)-2 cells, to microglia activated by aggregated Aβ can reverse the down-regulation of IGF-I expression, the up-regulation of TNF-α expression, and the failure to act as APCs (Butovsky et al., 2005). The significance of microglia for in-vivo neural cell renewal was demonstrated by enhanced neurogenesis in the rat dentate gyrus after injection of IL-4-activated microglia intracerebroventricularly and by the presence of IGF-I-expressing microglia in the dentate gyrus of rats kept in an enriched environment (Ziv et al., 2006). In rodents with acute or chronic EAE, injection of IL-4-activated microglia into the cerebrospinal fluid resulted in increased oligodendrogenesis in the spinal cord and improved clinical symptoms. The newly formed oligodendrocytes were spatially associated with microglia expressing MHC-II and IGF-I (Butovsky et al., 2006c).

In both Alzheimer's disease and AMD there are systemic components

Our first observation that systemic immune cells (in the form of T cells directed to certain self-antigens) can protect injured neurons from death came from studies in rodents showing that passive transfer of T cells specific to myelin basic protein reduces the loss of RGCs after a traumatic optic nerve injury (Moalem et al., 1999). We found that these T cells are also effective when directed to either cryptic or pathogenic epitopes of myelin basic protein, as well as to other myelin antigens or their epitopes (Mizrahi et al., 2002). These findings raised a number of critical questions. For example, are myelin antigens capable of protecting the nervous system from any type of acute or chronic insult? Is the observed neuroprotective activity of immune cells merely an anecdotal finding reflecting our experimental conditions, or does it point to the critical participation of the immune system in fighting off injurious conditions in the visual system and in the CNS in general? If the latter, does it mean that neurodegenerative diseases are systemic diseases? If so, can this finding be translated into a systemic therapy that would protect the brain, the eye, and the spinal cord?

In a series of experiments carried out over the last few years we have learned, firstly, that protective T cell response is a physiologically evoked response that might not be sufficient in severe insults or might not always be properly controlled. Moreover, we discovered that the specificity of such protective T cells depends on the site of the insult. Thus, for example, the protective effect of vaccination with myelin-associated antigens is restricted to injuries of the white matter, i.e., to myelinated axons (Mizrahi et al., 2002; Avidan et al., 2004; Schori et al., 2001). If the insult is to the retina, which contains no myelin, myelin antigens have no effect. Secondly, we observed that the injury-induced response of T cells reactive to specific self-antigens residing in the site of stress (eye or brain) is a spontaneous physiological response (Yoles et al., 2001). We then sought to identify the phenotype of the beneficial autoimmune T cells and to understand what determines the balance between a beneficial (neuroprotective) outcome of the T cell-mediated response to a CNS injury and a destructive effect causing autoimmune disease. We also examined ways of translating the beneficial response into a therapy for glaucoma. We found that in immune deficient animals the number of surviving RGCs following an insult in the eye, the spinal cord or the brain is significantly lower than in matched controls with an intact immune system, suggesting that the ability to withstand insult to the CNS depends on the integrity of the immune system and specifically on specific population within the immune system; those that recognize the site-specific self-antigens. Interestingly, the use of steroids caused significant loss of RGCs (Bakalash et al., 2003).

T cells specific to antigens residing in the site of damage help clean and heal

In order to be protective, the anti-self T cells should home to the site of damage and be locally activated. This is why only those antigens that are being presented at the site of lesion can be used for the vaccination. Once activated, the T cells provide a source of cytokines and growth factors that shape the resident eye sentinels cells – the microglia, so as to make them active defensible cells that the eye can tolerate. Namely, such activated microglia can take up glutamate, remove debris and produce growth factors while refraining from production of agents that are part of their killing mechanism (e.g. TNF-α) to which the eye, like the brain, has a low tolerance (Butovsky et al., 2005; Butovsky et al., 2001; Barouch & Schwartz, 2002; Moalem et al., 2000; Shaked et al., 2005). Such T cells are constitutively controlled by physiologically existing regulatory T cells that are themselves amenable to control upon need (Kipnis et al., 2004a; Kipnis et al., 2002).

Reference is made to copending International Patent Application No. PCT/IL2007/...... entitled "Activated myeloid cells for promoting tissue repair and detecting damaged tissue" filed by applicant at the Israel PCT Receiving Office (RO/IL) on the same date, the contents thereof being explicitly excluded from the scope of the present invention.

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SUMMARY OF THE INVENTION

It is an object of the present invention to provide an immune-based therapy for age-related macular degeneration.

In one aspect, the present invention relates to a method for treatment of age-related macular degeneration, which comprises causing T cells that produce IL-4 to accumulate in the eye of a patient in need, thereby halting or delaying progress of the macular degeneration.

In one embodiment, the accumulation of T cells in the eye is caused by administering to said patient an agent selected from the group consisting of:

(i) Copolymer-1, a Copolymer-1-related-peptide, or a Copolymer 1-related polypeptide;

(ii) IL-4;

(iii) dendritic cells, monocytes, bone marrow-derived myeloid cells or peripheral blood mononuclear cells activated by IL-4 ;

(iv) genetically engineered cells that produce IL-4;

(v) bone marrow-derived myeloid cells or peripheral blood-derived myeloid cells activated with IL-13 or with up to 20 ng/ml IFN-γ;

(vi) a pathogenic self-antigen associated with a T-cell-mediated specific autoimmune disease of the eye;

(vii) a peptide which sequence is comprised within the sequence of said pathogenic self-antigen of (vi) or a peptide obtained by modification of said peptide, which modification consists in the replacement of one or more amino acid residues of the peptide by different amino acid residues (hereinafter "modified peptide"), said modified peptide still being capable of recognizing the T-cell receptor recognized by the parent peptide but with less affinity;

(viii) a nucleotide sequence encoding a pathogenic self-antigen of (vi) or a peptide or a modified peptide of (vii) ;

(ix) T cells activated by an agent of (i), (vi) or (vii) ; and

(x) any combination of (i) – (ix).

In another aspect, the invention relates to the use of an agent as defined in (i) – (x) above for the preparation of a medicament for treatment of age-related macular degeneration.

BRIEF DESCRIPTION OF THE DRAWINGS

Figs. 1A-1D demonstrate that IL-4 can counteract the adverse effect of aggregated Aβ on microglial toxicity and promotion of neurogenesis in adult mouse neural progenitor cells. (**Fig. 1A**) *In-vitro* treatment paradigm. (**Fig. 1B**) Representative confocal microscopic images of neural progenitor cells (NPCs) expressing green fluorescent protein (GFP) and βIII-T (neuronal marker), co-cultured for 10 days without microglia (MG, control), or with untreated microglia, or with microglia that were pre-activated by aggregated $A\beta_{(1-40)}$ (5 μM) ($MG_{(A\beta 1-40)}$) for 48 h and subsequently activated with IFN-γ (10 ng/ml) ($MG_{(A\beta 1-40\,/\,IFN\gamma)}$) or with IL-4 (10 ng/ml) ($MG_{(A\beta 1-40\,/\,IL\text{-}4)}$) or with both IFN-γ (10 ng/ml) and IL-4 (10 ng/ml) ($MG_{(A\beta 1-40\,/\,IFN\gamma+IL\text{-}4)}$). Note, aggregated Aβ induced microglia to adopt an amoeboid morphology, but after IL-4 was added they exhibited a ramified structure. (**Fig. 1C**) Separate confocal images of NPCs co-expressing GFP and βIII-T adjacent to $CD11b^+$ microglia. (**Fig. 1D**) Quantification of cells double-labeled with GFP and βIII-T (expressed as a percentage of GFP^+ cells) obtained from confocal images. Results are of three independent experiments in replicate cultures; bars represent means ± SEM. Asterisks above bars denote the significance of differences relative to untreated (control) NPCs ($^*P < 0.05$; $^{***}P < 0.001$; two-tailed Student's t-test). Horizontal lines with P values above them show differences between the indicated groups (ANOVA).

Figs. 2A-2L show that Cop-1 vaccination leads to reduction in β-amyloid and counteracts loss of hippocampal neurons in the brains of transgenic Alzheimer's disease mice: key role of microglia. (**Fig. 2A**) Representative confocal microscopic images of brain hippocampal slices from non-transgenice (Tg), untreated-Tg-Alzheimer's disease (AD), and Cop-1-vaccinated Tg-AD mice stained for NeuN (mature neurons) and human Aβ. The non-Tg mouse shows no staining for human Aβ. The untreated-Tg-AD mouse shows an abundance of extracellular Aβ plaques, whereas in the Cop-1-treated Tg-AD mouse Aβ-immunoreactivity is low. Weak $NeuN^+$ staining is seen in the hippocampal CA1 and dentate gyrus regions of the untreated-Tg-AD mouse relative to its non-Tg littermate, whereas $NeuN^+$ staining in the Cop-1-vaccinated Tg-AD mouse is almost normal. (**Fig. 2B**) Staining for activated microglia using anti-CD11b antibodies. Images at low and high magnification show a high incidence of microglia double-stained for Aβ and CD11b in the CA1 and dentate gyrus regions of the hippocampus of an untreated-Tg-AD mouse, but only a minor presence of CD11b$^+$ microglia in the Cop-1-vaccinated Tg-AD mouse. Arrows indicate areas of high magnification, shown below. (**Fig. 2C**) CD11b$^+$ microglia, associated with an Aβ plaque, strongly expressing TNF-α in an untreated-Tg-AD mouse. (**Fig. 2D**) Staining for MHC-II (a marker of antigen presentation) in a cryosection taken from a Cop-1-vaccinated Tg-AD mouse in an area that stained positively for Aβ shows a high incidence of MHC-II$^+$ microglia and almost no TNF-α$^+$ microglia. (**Fig. 2E**) All MHC-II$^+$ microglia in a brain area that stained positively for Aβ (arrowheads) in a Cop-1-vaccinated Tg-AD mouse co-express CD11c (a marker of dendritic cells), but only a few CD11c$^+$/MHC-II$^+$ microglia are seen in a corresponding area in the brain of an untreated-Tg-AD mouse. (**Fig. 2F**) MHC-II$^+$ microglia in a Cop-1-vaccinated Tg-AD mouse co-expresses IGF-I. (**Fig. 2G**) CD3$^+$ T cells are seen in close proximity to an Aβ-plaque and (**Fig. 2H**) are associated with MHC-II$^+$ microglia. Boxed area shows high magnification of an immunological synapse between a T cell (CD3$^+$) and a microglial cell expressing MHC-II. (**Fig. 2I**) Histogram showing the total number of Aβ-plaques (in a 30-μm hippocampal slice). (**Fig. 2J**) Histogram showing staining for Aβ-immunoreactivity. Note the significant differences between Cop-1-vaccinated Tg-AD and untreated-Tg-AD mice, verifying the decreased presence of Aβ-plaques in the vaccinated mice. (**Fig. 2K**) Histogram showing a marked reduction in cells stained for CD11b, indicative of activated microglia and inflammation, in the Cop-1-vaccinated Tg-AD mice relative to untreated-Tg-AD mice. Note the increase in CD11b$^+$ microglia with age in the non-Tg littermates. (**Fig. 2L**) Histogram showing significantly more CD3$^+$ cells associated with an Aβ-plaque in Cop-1-vaccinated Tg-AD mice than in untreated-Tg-AD mice. Quantification of CD3$^+$ cells was analyzed from 30–50 plaques of each mouse tested in this study. Error bars indicate means ± SEM. $^*P < 0.05$, $^{***}P < 0.001$ versus non-Tg littermates (Student's *t*-test). The *P* values indicated in the figure represent a comparison of the groups as analyzed by ANOVA. All of the mice in this study were included in the analysis (6–8 sections per mouse).

Figs. 3A-3C show that Cop-1 vaccination induces microglia to express CD11c. (**Fig. 3A**) $CD11b^+$ microglia co-expressing CD11c surround an Aβ-plaque in Cop-1-vaccinated Tg-AD mice. All of the CD11c-expressing microglia are co-labeled for CD11b. Separate confocal channel is shown in right panel. (**Fig. 3B**) Histograms showing the number of $CD11b^+$ cells associated with Aβ-plaque. (**Fig. 3C**) Histograms showing quantification of $CD11c^+$ cells as a percentage of the total number of $CD11b^+$ and $CD11c^+$ cells associated with an Aβ-plaque. For this analysis, cells were counted surrounding 30–50 plaques in each mouse tested. Error bars represent means ± SEM. Asterisks above bars denote the significance of differences between the groups ($^{**}P < 0.01$; $^{***}P < 0.001$; two-tailed Student's *t*-test).

Figs. 4A-4D show that Cop-1 vaccination induces microglia to express CD11c: role of IL-4. (**Fig. 4A**) IL-4-activated microglia ($MG_{(IL\text{-}4)}$) induce CD11c expression in a primary culture of mouse microglia 5 days after activation. Untreated microglia ($MG_{(-)}$) express hardly any CD11c. (**Fig. 4B**) Effect of IL-4 (in terms of morphology and CD11c expression) on microglia pretreated for 3 days with aggregated $A\beta_{(1-40)}$ ($MG_{(A\beta)}$) and assessed 10 days later compared to IL-4 treatment for 10 days without pre-exposure to Aβ. Note that dendritic-like morphology was adopted upon addition of IL-4 to the Aβ-pretreated microglia only, whereas CD11c expression was induced by IL-4 both with and without Aβ pretreatment. (**Fig. 4C**) Quantitative analysis of microglial expression of $CD11c^+$ microglia (expressed as a percentage of IB-4-labeled microglia) and of CD11c intensity per cell, both expressed as a function of time in culture with or without IL-4. (**Fig. 4D**) Quantitative analysis of CD11c expression (calculated as a percentage of IB-4-labeled microglia) by the cultures shown in (**Fig. 4B**). Results are of three independent experiments in replicate cultures; bars represent means ± SEM. Asterisks above bars denote the significance of differences relative to untreated microglia at each time point ($^{***}P < 0.001$; two-tailed Student's *t*-test).

Figs. 5A-5B show engulfment of aggregated Aβ by activated microglia. Microglia were treated with IL-4 (10 ng/ml) 24 h after seeding ($MG_{(IL\text{-}4)}$) or were left untreated for 48 h ($MG_{(-)}$). The media were then replaced by a labeling medium (DMEM containing 10 mg/ml bovine serum albumin), and aggregated $A\beta_{(1-40)}$ was added (5 μg/ml) for 1 h. Following incubation the cultures were fixed and immunostained with antibodies directed to human Aβ and co-stained for microglia (IB-4). (**Fig. 5A**) Confocal photomicrographs. (**Fig. 5B**) Quantitative analysis expressed as intensity per cell. Results of one of two experiments, each containing eight replicates (20–30 cells per replicate) per group are presented (means ± SD).

Figs. 6A-6E depict enhanced neurogenesis induced by Cop-1 vaccination in the hippocampal dentate gyri of adult transgenic (Tg) AD mice. Three weeks after the first Cop-1 vaccination, mice in each experimental group were injected i.p. with 5-bromo-2'-deoxyuridine (BrdU) twice daily for 2.5 days. Three weeks after the last injection their brains were excised and the hippocampi analyzed for BrdU, DCX (a marker of early differentiation of the neuronal lineage), and NeuN (a marker of mature neurons). (**Figs. 6A-6C**) Histograms showing quantification of the proliferating cells ($BrdU^+$). (**Fig. 6A**) Newly formed mature neurons ($BrdU^+/NeuN^+$) (**Fig. 6B**), and all pre-mature (DCX^+-stained) neurons (**Fig. 6C**). Numbers of $BrdU^+$, $BrdU^+/NeuN^+$ and DCX^+ cells per dentate gyrus (DG), calculated from six equally spaced coronal sections (30 μm) from both sides of the brains of all the mice tested in this study. Error bars represent means ± SEM. Asterisks above bars denote the significance of differences relative to non-Tg littermates ($^{**}P < 0.01$; $^{***}P < 0.001$; two-tailed Student's *t*-test). Horizontal lines with *P* values above them show differences between the indicated groups (ANOVA). (**Fig. 6D**) Representative confocal microscopic images of the dentate gyrus showing immunostaining for BrdU/DCX/NeuN in a Cop-1-vaccinated Tg-AD mouse and in a non-Tg littermate relative to that in an untreated-Tg-AD mouse. (**Fig. 6E**) Branched DCX^+ cells are found near MHC-II^+ microglia located in the subgranular zone (SGZ) of the hippocampal dentate gyrus of a Cop-1-vaccinated Tg-AD mouse.

Figs. 7A-7B show that Cop-1 vaccination counteracts cognitive decline in transgenic (Tg) AD mice. Hippocampus-dependent cognitive activity was tested in the Morris water maze (MWM). (**Figs. 7A-7B**) Cop-1-vaccinated Tg-AD mice (*diamond; n = 6*) showed significantly better learning/memory ability than untreated-Tg-AD mice (*square; n = 7*) during the acquisition and reversal. Untreated-Tg-AD mice showed consistent and long-lasting impairments in spatial memory tasks. In contrast, performance of the MWM test by the Cop-1-vaccinated Tg-AD mice was rather similar, on average, to that of their age-matched naïve non-Tg littermates (*triangle; n = 6*) (3-way ANOVA, repeated measures: groups, df (2,16), $F = 22.3$, $P < 0.0002$; trials, df (3,48), $F = 67.9$, $P < 0.0001$; days, df (3,48), $F = 3.1$, $P < 0.035$, for the acquisition phase; and groups, df (2,16), $F = 14.9$, $P < 0.0003$; trials, df (3,48), $F = 21.7$, $P < 0.0001$; days, df (1,16), $F = 16.9$, $P < 0.0008$, for the reversal phase).

Figs. 8A-8D show a time course of CD11c expression in microglia activated by IFN-γ and IL-4. (**Fig. 8A**) Microglia were treated with IFN-γ (10 ng/ml; $MG_{(IFN-\gamma)}$) or IL-4 (10 ng/ml; $MG_{(IL-4)}$) for 1, 3, 5, 10 and 18 days. Untreated microglia ($MG_{(-)}$)were used as controls. (**Fig. 8B**) Confocal images of microglia, identified by staining for IB4, immunolabeled for βIII-T, and CD11c after 5 days of treatment. $MG_{(-)}$ did not express CD11c. After exposure to IFN-γ or IL-4 the microglia expressed CD11c and exhibited their characteristic morphology. (**Fig. 8C**) Co-expression of βIII-T and CD11c in microglia activated with IFN-γ (10 ng/ml) for 5 days (IB4/βIII-T/CD11c). Note, confocal channels are presented separately. (**Fig. 8D**) Quantitative analysis of the numbers of $CD11c^+$ microglia (expressed as a percentage of $IB4^+$ (microglia marker) cells) were examined in all treatments at all time points. Results are of four independent experiments with duplicate or triplicate wells; bars represent means ± SEM. Asterisks above bars denote the significance of differences relative to $MG_{(-)}$ ($^*P$

< 0.05; $^{**}P < 0.01$; $^{***}P < 0.001$; two-tailed Student's *t*-test). Horizontal lines with *P* values above them show differences between the indicated groups (ANOVA).

DETAILED DESCRIPTION OF THE INVENTION

In searching for a prospect for a T-cell-based vaccination for treatment of AMD, the following considerations may be relevant.

In general, the brain, like the retina, is considered to be immune privileged in the sense that the blood-brain barrier resists passive deposition of antibodies and reduces the recruitment of antigen-specific lymphocytes (Streilein et al., 1992). Paradoxically, antigen-specific immunity might actually function to protect against degenerative diseases. Recently, it was shown in a Alzheimer's disease mouse model that immunization with the abnormal amyloid, or passive administration of antibodies against the abnormal protein, greatly reduced the quantity of deposition in the brain of the genetically modified mice, and improved their performance in laboratory tests of memory and cognitive function (e.g. Morgan et al., 2000). The mechanisms are unclear, but may be related to enhanced phagocytosis, neutralization of toxic molecules, or interference with amyloid fibril aggregation. In Alzheimer's disease however, patients may be immunologically tolerant to amyloid, preventing protective autoimmunization to the abnormally processed protein and thus developing autoimmune encephalomyelitis (Furlan et al., 2003).

Studies from our the laboratory over the last few years have shown that recovery from CNS injury is critically dependent on the well-controlled activity of T cells directed to specific CNS autoantigens (Moalem et al., 1999; Yoles et al., 2001; Kipnis et al., 2002). After homing to the site of damage, these autoreactive T cells evidently regulate microglia in a way that renders them supportive of neuronal survival and neural tissue repair (Butovsky et al., 2005; Schwartz et al., 2006; Butovsky et al., 2001; Shaked et al., 2005).

Our results herein argue in favor of the use of a myelin-related antigen such as Cop-1, but not an Aβ peptide, as a T cell-based therapy for AMD. Even if any T cells expressing T-cell receptors for drusen-associated peptides such as Aβ were to home to the site of a CNS lesion (Monsonego et al., 2006), it is unlikely that they would encounter their relevant APCs there, and they would therefore not be able to become locally activated.

On the other hand, myelin-presenting microglia, with which myelin-specific T cells can readily hold a dialog, are likely to be present at the damaged sites. Myelin-related antigens, or antigens that are weakly cross-reactive with myelin (such as Cop-1), are therefore likely to be the antigens of choice for therapeutic vaccination (Avidan et al., 2004). T cells activated by these antigens will then home to the CNS and, upon encountering their relevant APCs there, become locally activated to supply the cytokines and growth factors in order to switch the phenotype of the harmful microglia (activated by aggregated Aβ; Butovsky et al., 2005) into microglia with dendritic-like characteristics. The resulting immunological synapse between T cells and microglia will then create a supportive niche for cell renewal by promoting neurogenesis from the pool of adult stem cells (Butovsky et al., 2006a).

Our results indicate that T cells constitute the immune-based therapy of choice for AMD. This does not preclude the potential benefit of antibodies as a supplementary therapy as shown in anima; model of Alzheimer disease with antibodies against Aβ peptide (Bard et al., 2000). Moreover, the T cells can function as a mini-factory capable of producing a variety of compounds, including cytokines and neurotrophic factors (Ziemssen et al., 2002). Above all, they represent a physiological system of maintenance and repair that might help to counteract the age-related conditions leading to brain senescence.

In developing the immune-based therapy for AMD according to the present invention we thus took into consideration the lessons from neurodegenerative diseases with similar pathogenicity.

Formation of extracellular deposits consisting of misfolded protein is the hallmark of many neurodegenerative diseases. The accumulation of amyloid in drusen and Alzheimer's disease and the presence of activated microglia as inflammatory mediators in both neurodegenerative conditions, suggests a possible common chronic inflammatory pathway in AMD and Alzheimer's disease. In the context of Alzheimer's disease we have recently demonstrated that aggregated β-amyloid (Aβ) activates microglia to acquire a phenotype which is reminiscence of that activated by microorganisms. Although such microglia/macrophage can act as phagocytic cells their overall activity is cytotoxic and can hardly be tolerated by the brain. As a result, microglia activated by Aβ rather than help the suffering tissue further contribute to the chaos. In the present invention, we explore possible immune-based therapy to modulate microglia activity in Alzheimer's disease and AMD with the target of maintaining their phagocytic activity while conferring their ability to support cell survival and renewal.

As shown herein, aggregated Aβ induces microglia to become cytotoxic and block neurogenesis from adult rodent neural progenitor cells (NPCs). Addition of IL-4, a cytokine derived from T-helper (Th)2 cells, to microglia activated by Aβ can reverse the impediment, the down-regulation of IGF-I, the up-regulation of TNF-α, and the failure to act as APCs. Using Alzheimer's disease double-transgenic mice expressing mutant human genes encoding presenilin 1 and chimeric mouse/human amyloid precursor protein, we show that switching of microglia phenotype into professional APCs producing IGF-I, achieved here by a T cell-based vaccination with Copolymer-1, resulted in reduction of amyloid loads and the induction of neuronal survival and neurogenesis.

The present invention thus relates to a method for treatment of age-related macular degeneration (AMD), which comprises causing T cells that produce IL-4 to accumulate in the eye of an individual in need, thereby halting or delaying progress of the macular degeneration. This effect can be affected by several self antigens and cytokine activated cells.

In one embodiment, the agents that can cause T cells producing IL-4 to accumulate in the eye include, without being limited to:

(i) Copolymer-1, a Copolymer-1-related-peptide, or a Copolymer 1-related polypeptide;

(ii) IL-4;

(iii) dendritic cells, monocytes, bone marrow-derived myeloid cells or peripheral blood mononuclear cells activated by IL-4 ;

(iv) genetically engineered cells that produce IL-4;

(v) bone marrow-derived myeloid cells or peripheral blood-derived myeloid cells activated with IL-13 or with up to 20 ng/ml IFN-γ;

(vi) a pathogenic self-antigen associated with a T-cell-mediated specific autoimmune disease of the eye;

(vii) a peptide which sequence is comprised within the sequence of said pathogenic self-antigen of (vi) or a peptide obtained by modification of said peptide, which modification consists in the replacement of one or more amino acid residues of the peptide by different amino acid residues (hereinafter “modified peptide”), said modified peptide still being capable of recognizing the T-cell receptor recognized by the parent peptide but with less affinity;

(viii) a nucleotide sequence encoding a pathogenic self-antigen of (vi) or a peptide or a modified peptide of (vii) ;

(ix) T cells activated by an agent of (i), (vi) or (vii); and

(x) any combination of (i) – (ix).

In one preferred embodiment, the agent is Copolymer 1, a Copolymer 1-related peptide or a Copolymer 1-related polypeptide.

For the purpose of the present invention, "Copolymer 1 or a Copolymer 1-related peptide or polypeptide" is intended to include any peptide or polypeptide, including a random copolymer that cross-reacts functionally with MBP and is able to compete with MBP on the MHC class II in the antigen presentation.

The Cop 1 or a Cop 1-related peptide or polypeptide is represented by a random copolymer consisting of a suitable ratio of a positively charged amino acid such as lysine or arginine, in combination with a negatively charged amino acid (preferably in a lesser quantity) such as glutamic acid or aspartic acid, optionally in combination with a non-charged neutral amino acid such as alanine or glycine, serving as a filler, and optionally with an amino acid adapted to confer on the copolymer immunogenic properties, such as an aromatic amino acid like tyrosine or tryptophan. Such copolymers are disclosed, for example, in WO 00/05250, the entire contents of which are herewith incorporated herein by reference.

More specifically, the Copolymer 1 or a Copolymer 1-related peptide or polypeptide is a copolymer selected from the group consisting of random copolymers comprising one amino acid selected from each of at least three of the following groups: (a) lysine and arginine; (b) glutamic acid and aspartic acid; (c) alanine and glycine; and (d) tyrosine and tryptophan. The amino acids may be L- or D-amino acids or mixtures thereof. The present invention contemplates the use of copolymers containing both D- and L-amino acids, as well as copolymers consisting essentially of either L- or D-amino acids.

In one embodiment of the invention, the copolymer contains four different amino acids, each from a different one of the groups (a) to (d).

In a more preferred embodiment, the agent is Copolymer 1, composed of a mixture of random polypeptides consisting essentially of the amino acids L-glutamic acid (E), L-alanine (A), L-tyrosine (Y) and L-lysine (K) in an approximate ratio of 1.5:4.8:1:3.6, having a net overall positive electrical charge and of a molecular weight from about 2 KDa to about 40 KDa.

In one preferred embodiment, the Cop 1 has average molecular weight of about 2 KDa to about 20 KDa, more preferably of about 4,7 KDa to about 13 K Da, still more preferably of about 4 KDa to about 8.6 KDa, of about 5 KDa to 9 KDa, or of about 6.25 KDa to 8.4 KDa. In another preferred embodiment, the Cop 1 has average molecular weight of about 13 KDa to about 20 KDa, more preferably of about 13,5 KDa to about 18 KDa, with an average of about 15 KDa to about 16 KD, preferably of 16kDa. Other average molecular weights for Cop 1, lower than 40 KDa, are also encompassed by the present invention. Copolymer 1 of said molecular weight ranges can be prepared by methods known in the art, for example by the processes described in U.S. Patent No. 5,800,808, the entire contents of which are hereby incorporated by reference in the entirety. The Copolymer 1 may be a polypeptide comprising from about 15 to about 100, preferably from about 40 to about 80, amino acids in length.

In one more preferred embodiment of the invention, the agent is Cop 1 in the form of its acetate salt known under the generic name glatiramer acetate or its trade name Copaxone® (a trademark of Teva Pharmaceutical Industries Ltd., Petach Tikva, Israel). As used herein in the application, the terms "Cop 1", "Copolymer 1", "glatiramer acetate" and "GA" are used interchangeably.

In another embodiment, the Copolymer 1-related peptide is a random copolymer of 4 amino acids in which one or more of the following substitutions is made: aspartic acid for glutamic acid, glycine for alanine, arginine for lysine, and tryptophan for tyrosine, which is expected to have the same activity of Copolymer.

In another embodiment of the invention, the Cop 1-related peptide or polypeptide is a copolymer of three different amino acids each from a different one of three groups of the groups (a) to (d). These copolymers are herein referred to as terpolymers. In one embodiment, the terpolymer contains tyrosine (Y), alanine (A), and lysine (K), hereinafter designated YAK, in which the average molar fraction of the amino acids can vary: Y, A and K can be present in a mole fraction of about 0.05-0.250, 0.3 - 0.6; and 0.1-0.5, respectively, more preferably, the molar ratios of Y, A and K are about 0.10:0.54:0.35, respectively. It is possible to substitute arginine for lysine, glycine for alanine, and/or tryptophan for tyrosine.

In another embodiment, the terpolymer contains tyrosine (Y), glutamic acid (E), and lysine (K), hereinafter designated YEK, in which the average molar fraction of the amino acids can vary: E, Y and K can be present in a mole fraction of about 0.005 - 0.300, 0.005-0.250, and 0.3-0.7, respectively. More preferably, the molar ratios of E, Y and K are about 0.26:0.16:0.58, respectively. It is possible to substitute aspartic acid for glutamic acid, arginine for lysine, and/or tryptophan for tyrosine.

In another embodiment, the terpolymer contains lysine (K), glutamic acid (E), and alanine (A), hereinafter designated KEA, in which the average molar fraction of the amino acids can vary: E, A and K van be present in a mole fraction of about 0.005-0.300, 0.005-0.600, and 0.2 - 0.7, respectively. More preferably, the molar ratios of E, A and K are about 0.15:0.48:0.36, respectively. It is possible to substitute aspartic acid for glutamic acid, glycine for alanine, and/or arginine for lysine.

In a further embodiment, the terpolymer contains tyrosine (Y), glutamic acid (E), and alanine (A0, hereinafter designated YEA, in which the average molar fraction of the amino acids can vary: Y, E and A can be present in a mole fraction of about 0.005-0.250, 0.005-0.300, and 0.005-0.800, respectively. More preferably, the molar ratios of E, A, and Y are about 0.21: 0.65:0.14, respectively. It is possible to substitute tryptophan for tyrosine, aspartic acid for glutamic acid, and/or glycine for alanine.

The average molecular weight of the terpolymers YAK, YEK, KEA and YEA can vary between about 2 KDa to 40 KDa, preferably between about 3 KDa to 35 KDa, more preferably between about 5 KDa to 25 KDa.

Copolymer 1 and related peptides and polypeptides may be prepared by methods known in the art, for example, by the process disclosed in U.S. Patent 3,849,550. The molecular weight of the copolymers can be adjusted during polypeptide synthesis or after the copolymers have been made. To adjust the molecular weight during polypeptide synthesis, the synthetic conditions or the amounts of amino acids are adjusted so that synthesis stops when the polypeptide reaches the approximate length that is desired. After synthesis, polypeptides with the desired molecular weight can be obtained by any available size selection procedure, such as chromatography of the polypeptides on a molecular weight sizing column or gel, and collection of the molecular weight ranges desired. The copolymers can also be partially hydrolyzed to remove high molecular weight species, for example, by acid or enzymatic hydrolysis, and then purified to remove the acid or enzymes.

In one embodiment, the copolymers with a desired molecular weight may be prepared by a process, which includes reacting a protected polypeptide with hydrobromic acid to form a trifluoroacetyl-polypeptide having the desired molecular weight profile. The reaction is performed for a time and at a temperature that is predetermined by one or more test reactions. During the test reaction, the time and temperature are varied and the molecular weight range of a given batch of test polypeptides is determined. The test conditions that provide the optimal molecular weight range for that batch of polypeptides are used for the batch. Thus, a trifluoroacetyl-polypeptide having the desired molecular weight profile can be produced by a process, which includes reacting the protected polypeptide with hydrobromic acid for a time and at a temperature predetermined by test reaction. The trifluoroacetyl-polypeptide with the desired molecular weight profile is then further treated with an aqueous piperidine solution to form a low toxicity polypeptide having the desired molecular weight. In one embodiment, a test sample of protected polypeptide from a given batch is reacted with hydrobromic acid for about 10-50 hours at a temperature of about 20-28°C. The best conditions for that batch are determined by running several test reactions. For example, in one embodiment, the protected polypeptide is reacted with hydrobromic acid for about 17 hours at a temperature of about 26°C.

As binding motifs of Cop 1 to MS-associated HLA-DR molecules are known (Fridkis-Hareli et al, 1999), polypeptides derived from Cop 1 having a defined sequence can readily be prepared and tested for binding to the peptide binding groove of the HLA-DR molecules as described in the Fridkis-Hareli et al (1999) publication.

Examples of such peptides are those disclosed in WO 00/05249 and WO 00/05250, the entire contents of which are hereby incorporated herein by reference, and include the peptides of SEQ ID NOs. 1-32 hereinbelow.

SEQ ID NO:1	AAAYAAAAAAKAAAA
SEQ ID NO:2	AEKYAAAAAAKAAAA
SEQ ID NO:3	AKEYAAAAAAKAAAA
SEQ ID NO:4	AKKYAAAAAAKAAAA
SEQ ID NO:5	AEAYAAAAAAKAAAA
SEQ ID NO:6	KEAYAAAAAAKAAAA
SEQ ID NO:7	AEEYAAAAAAKAAAA
SEQ ID NO:8	AAEYAAAAAAKAAAA
SEQ ID NO:9	EKAYAAAAAAKAAAA
SEQ ID NO:10	AAKYEAAAAAKAAAA
SEQ ID NO:11	AAKYAEAAAAKAAAA
SEQ ID NO:12	EAAYAAAAAAKAAAA
SEQ ID NO:13	EKKYAAAAAAKAAAA
SEQ ID NO:14	EAKYAAAAAAKAAAA
SEQ ID NO:15	AEKYAAAAAAAAAAA
SEQ ID NO:16	AKEYAAAAAAAAAAA
SEQ ID NO:17	AKKYEAAAAAAAAAA
SEQ ID NO:18	AKKYAEAAAAAAAAA
SEQ ID NO:19	AEAYKAAAAAAAAAA
SEQ ID NO:20	KEAYAAAAAAAAAAA
SEQ ID NO:21	AEEYKAAAAAAAAAA
SEQ ID NO:22	AAEYKAAAAAAAAAA
SEQ ID NO:23	EKAYAAAAAAAAAAA
SEQ ID NO:24	AAKYEAAAAAAAAAA
SEQ ID NO:25	AAKYAEAAAAAAAAA
SEQ ID NO:26	EKKYAAAAAAAAAAA
SEQ ID NO:27	EAKYAAAAAAAAAAA
SEQ ID NO:28	AEYAKAAAAAAAAAA
SEQ ID NO:29	AEKAYAAAAAAAAAA
SEQ ID NO:30	EKYAAAAAAAAAAAA
SEQ ID NO:31	AYKAEAAAAAAAAAA
SEQ ID NO:32	AKYAEAAAAAAAAAA

Such peptides of SEQ ID Nos: 1-32 and other similar peptides derived from Cop 1 would be expected to have similar activity as Cop 1. Such peptides, and other similar peptides, are also considered to be within the definition of Cop 1-related peptides or polypeptides and their use is encompassed by the present invention as well as other synthetic amino acid copolymers such as the random four-amino acid copolymers described by Fridkis-Hareli et al., 2002 (as candidates for treatment of multiple sclerosis), namely copolymers (14-, 35- and 50-mers) containing the amino acids phenylalanine, glutamic acid, alanine and lysine (poly FEAK), or tyrosine, phenylalanine, alanine and lysine (poly YFAK), and any other similar copolymer to be discovered that can be considered a universal antigen similar to Cop 1.

In another preferred embodiment of the present invention, the agent that causes T cells that produce IL-4 to accumulate in the eye is IL-4, preferably human recombinant IL-4, that can be administered nasally.

In a further embodiment, the agent is IL-4 activated dendritic cells, IL-4 activated monocytes, IL-4 activated bone marrow-derived myeloid cells or IL-4 activated peripheral blood mononuclear cells (PBMCs). In this regard, IL-13 have the same effect as IL-4, because it is well established in the field of cytokines that IL-4 and IL-13 can utilize a common receptor and share many actions such as B-cell activation and suppression of Th-1 cells.

In an additional embodiment, the agent is bone marrow-derived myeloid cells or peripheral blood-derived myeloid cells activated with IL-13 or with a narrow concentration range of IFN-γ, more preferably up to 20 ng/ml IFN-γ.

In another embodiment, the agent is a mammalian pathogenic self-antigen associated with a T-cell-mediated specific autoimmune disease of the eye such as, but not limited to, a pathogenic uveitogenic antigen selected from mammalian interphotoreceptor retinoid-binding protein (IRBP), S-antigen (S-Ag), or rhodopsin. The mammalian uveitogenic antigen is preferably a human IRBP (SEQ ID NO: 33) or a bovine IRBP (SEQ ID NO: 34), a human S-Ag (SEQ ID NO: 35) or a bovine S-Ag (SEQ ID NO: 36), or human rhodopsin (SEQ ID NO: 37).

In still another embodiment, the agent is a peptide which sequence is comprised within the sequence of said pathogenic self-antigen; a peptide obtained by modification of said peptide, which modification consists in the replacement of one or more amino acid residues of the peptide by different amino acid residues (hereinafter "modified peptide"), said modified peptide still being capable of recognizing the T-cell receptor recognized by the parent peptide but with less affinity; or a nucleotide sequence encoding said pathogenic self-antigen, said peptide or said modified peptide.

In one embodiment, the agent is: (a) a peptide which sequence is comprised within the sequence of bovine IRBP (SEQ ID NO: 34); (b) a modified peptide as defined above obtained by modification of the peptide of (a); or (c) a nucleotide sequence encoding human or bovine IRPB, a peptide of (a), or a modified peptide of (b).

The peptide which sequence is comprised within the sequence of bovine IRBP (SEQ ID NO: 34) may be the peptide R16 (sequence 1177–1191 of IRBP), ADGSSWEGVGVVPDV (SEQ ID NO:38); the peptide PTARSVGAADGSSWEGVGVVPDV (SEQ ID NO:39); or the peptide HVDDTDLYLTIPTARSVGAADGS (SEQ ID NO:40).

In another embodiment, the agent is: (a) a peptide which sequence is comprised within the sequence of bovine S-Ag (SEQ ID NO:36); (b) a modified peptide as defined above obtained by modification of the peptide of (a); or (c) a nucleotide sequence encoding human or bovine S-Ag, a peptide of (a), or a modified peptide of (b).

The peptide (a) which sequence is comprised within the sequence of bovine S-Ag may be the peptide G-8 (sequence 347–354 of S-Ag) of the sequence TSSEVATE (SEQ ID NO:41); the peptide M-8 (sequence 307–314 of S-Ag), DTNLASST (SEQ ID NO:42; or the peptides of the sequences:

DTNLASSTIIKEGIDKTV	(SEQ ID NO:43);
VPLLANNRERRGIALDGKIKHE	(SEQ ID NO:44);
TSSEVATEVPFRLMHPQPED	(SEQ ID NO:45);
SLTKTLTLVPLLANNRERRG	(SEQ ID NO:46);
SLTRTLTLLPLLANNRERAG	(SEQ ID NO:47);
KEGIDKTVMGILVSYQIKVKL	(SEQ ID NO:48); and
KEGIDRTVLGILVSYQIKVKL	(SEQ ID NO:49).

The modified peptide (c) derived from bovine S-Ag may be the G-8 analog, TSSEAATE (SEQ ID NO:50) or the M-8 analog, DTALASST (SEQ ID NO:51).

In another embodiment, the agent is a nucleotide sequence encoding a pathogenic self-antigen associated with a T-cell-mediated specific autoimmune disease of the eye, or a peptide or a modified peptide derived therefrom as defined herein.

In a further embodiment, the agent is T cells activated by Copolymer 1, or by a pathogenic self-antigen associated with a T-cell-mediated specific autoimmune disease of the eye, a peptide or a modified peptide derived therefrom as defined herein.

In yet a further embodiment, the agent is any combination of the agents defined above.

The invention further relates to the use of an agent selected from the agents defined herein for treatment of age-related macular degeneration or for the manufacture of a medicament for treatment of age-related macular degeneration.

When the agent is activated cells as described above, the cells can be preferably autologous or they can be from a matched donor and are preferably administered intravenously.

In one preferred embodiment, the agent is T cells activated by Copolymer 1, which can be prepared close to the administration step or cell banks can be established to store Copolymer 1-sensitized T cells for treatment of individuals at a later time, as needed. Autologous T cells may be obtained from the individual and allogeneic or semi-allogeneic T cells may obtained from a bank of stored T cells of each of the most common MHC-class II types are present. The patient is preferably treated with its autologous stored T cells, but if autologous T cells are not available, then cells should be used which share an MHC type II molecule with the patient, and these would be expected to be operable in that patient. The T cells are preferably stored in an activated state after exposure to Copolymer 1. However, the T cells may also be stored in a resting state and activated with Copolymer 1 once they are thawed and prepared for use.

The T cell lines are prepared in any way that is well known in the art. The cell lines of the bank are preferably cryopreserved. Once the cells are thawed, they are preferably cultured prior to injection in order to eliminate non-viable cells. During this culturing, the T cells can be activated or reactivated using the Copolymer 1 antigen as used in the original activation. Alternatively, activation may be achieved by culturing in the presence of a mitogen, such as phytohemagglutinin (PHA) or concanavalin A (preferably the former). This will place the cells into an even higher state of activation.

The bone marrow-derived myeloid cells for use in the present invention can be obtained from autologous or donor's peripheral blood or bone marrow and processed by techniques well-known in the art. The donor should be a matched, namely HLA-matched, donor. Once obtained, the myeloid cells may be cultured until they multiply to the level needed for transplant back into the patient and are then activated with the cytokine (IL-4, IL-13 or IFN-γ) for the time necessary to upregulate CD11c expression. For example, activation with up to 20 ng/ml IFN-γ may take 2-3 days until the peak of CD11c expression is reached.

In the examples, $CD11c^+$ microglia cells are described. Microglia are immune cells of the CNS that are derived from myeloid progenitor cells, which come from the bone marrow. Thus, microglia are the resident CNS cells whereas the bone marrow-derived myeloid cells are the infiltrating cells.

Pharmaceutical compositions/medicaments for use in accordance with the present invention may be formulated in conventional manner using one or more pharmaceutically/physiologically acceptable carriers or excipients, depending on the agent used. The carrier(s) must be "acceptable" in the sense of being compatible with the other ingredients of the composition and not deleterious to the recipient thereof.

Methods of administration and doages will depend on the agent used and include, but are not limited to, parenteral, e.g., intravenous, intraperitoneal, intramuscular, subcutaneous, mucosal, e.g., oral, intranasal, buccal, vaginal, rectal, or intraocular, intrathecal, topical and intradermal routes, with or without adjuvant. Administration can be systemic or local.

The dosage of the agent to be administered will be determined by the physician according to the agent, the age of the patient and stage of the disease. For example, for Cop 1, the dosage may be chosen from a range of 1-80 mg, preferably 20 mg, although any other suitable dosage is encompassed by the invention. The treatment can be carried out by administration of repeated doses at suitable time intervals, according to the stage of the disease, the age and condition of the patient. In one embodiment, Cop 1 may be administered daily. In another preferred embodiment, the administration may be made according to a regimen suitable for immunization, for example, at least once a week, once a month or at least once every 2 or 3 months, or less frequently, but any other suitable interval between the immunizations is envisaged by the invention according to the condition of the patient.

When the agent is genetically engineered cells that produce IL-4, the cells are preferably engineered bone marrow–derived dendritic cells (DCs) that express IL-4, which may be obtained as described by Morita et al., 2001. Since DCs are specialized APCs that migrate from the periphery to lymphoid tissues, where they activate and regulate T cells, genetic modification of DCs to express immunoregulatory cytokines such as IL-4 provides a new immunotherapeutic strategy for treatment of AMD and other diseases.

The invention will now be illustrated by the following non-limiting examples.

EXAMPLES

Materials and Methods

(i) Animals. Neonatal (P0-P1) mice, inbred adult male C57Bl/6J mice (8-10 weeks) were supplied by the Animal Breeding Center, Weizmann Institute of Science, Rehovot, Israel.

(ii) Neural progenitor cell culture. Coronal sections (2 mm thick) of tissue containing the subventricular zone of the lateral ventricle were obtained from the brains of adult C57Bl/6J mice. The tissue was minced and then incubated for digestion at 37°C, 5% CO_2 for 45 min in Earle's balanced salt solution containing 0.94 mg/ml papain (Worthington, Lakewood, NJ) and 0.18 mg/ml of L-cysteine and EDTA. After centrifugation at 110 × *g* for 15 min at room temperature, the tissue was mechanically dissociated by pipette trituration. Cells obtained from single-cell suspensions were plated (3500 cells/cm^2) in 75-cm^2 Falcon tissue-culture flasks (BD Biosciences, San Diego, CA), in neural stem/progenitor cell (NPC)-culturing medium [Dulbecco's modified Eagles's medium (DMEM)/F12 medium (Gibco/Invitrogen, Carlsbad, CA) containing 2 mM L-glutamine, 0.6% glucose, 9.6 μg/ml putrescine, 6.3 ng/ml progesterone, 5.2 ng/ml sodium selenite, 0.02 mg/ml insulin, 0.1 mg/ml transferrin, 2 μg/ml heparin (all from Sigma-Aldrich, Rehovot, Israel), fibroblast growth factor-2 (human recombinant, 20 ng/ml), and epidermal growth factor (human recombinant, 20 ng/ml; both from Peprotech, Rocky Hill, NJ)]. Spheres were passaged every 4–6 days and replated as single cells. Green fluorescent protein (GFP)-expressing NPCs were obtained as previously described (Pluchino et al., 2003).

(iii) Primary microglial culture. Brains from neonatal (P0–P1) C57Bl/6J mice were stripped of their meninges and minced with scissors under a dissecting microscope (Zeiss, Stemi DV4, Germany) in Leibovitz-15 medium (Biological Industries, Kibbutz Beit Ha-Emek, Israel). After trypsinization (0.5% trypsin, 10 min, 37°C/5% CO_2), the tissue was triturated. The cell suspension was washed in culture medium for glial cells [DMEM supplemented with 10% fetal calf serum (FCS; Sigma-Aldrich, Rehovot), L-glutamine (1 mM), sodium pyruvate (1 mM), penicillin (100 U/ml), and streptomycin (100 mg/ml)] and cultured at 37°C/5% CO_2 in 75-cm^2 Falcon tissue-culture flasks (BD Biosciences) coated with poly-D-lysine (PDL) (10 mg/ml; Sigma-Aldrich, Rehovot) in borate buffer (2.37 g borax and 1.55 g boric acid dissolved in 500 ml sterile water, pH 8.4) for 1 h, then rinsed thoroughly with sterile, glass-distilled water. Half of the medium was changed after 6 h in culture and every 2nd day thereafter, starting on day 2, for a total culture time of 10–14 days. Microglia were shaken off the primary mixed brain glial cell cultures (150 rpm, 37°C, 6 h) with maximum yields between days 10 and 14, seeded (10^5 cells/ml) onto PDL-pretreated 24-well plates (1 ml/well; Corning, New York, NY), and grown in culture medium for microglia [RPMI-1640 medium (Sigma-Aldrich, Rehovot) supplemented with 10% FCS, L-glutamine (1 mM), sodium pyruvate (1 mM), β-mercaptoethanol (50 mM), penicillin (100 U/ml), and streptomycin (100 mg/ml)]. The cells were allowed to adhere to the surface of a PDL-coated culture flask (30 min, 37°C/5% CO_2), and non-adherent cells were rinsed off.

(iv) Immunocytochemistry and Immunohistochemistry. Primary antibodies: *Bandeiraea simplicifolia* isolectin B4 (IB-4; 1:50; Sigma-Aldrich, Rehovot); mouse anti-β-tubulin (anti-βIII-T) isoform C-terminus antibodies (1:500; Chemicon, Temecula, CA), rat anti-CD11b (MAC1; 1:50; BD–Pharmingen, Franklin Lakes, NJ), hamster anti-CD11c (1:100; eBioscience, San Diego, CA), rat anti-MHC-II Abs (clone IBL-5/22; 1:50), mouse anti-Aβ (human amino-acid residues 1–17; clone 6E10; Chemicon), rat anti-BrdU (1:200; Oxford Biotechnology, Kidlington, Oxfordshire, UK), goat anti-doublecortin (anti-DCX) (1:400; Santa Cruz Biotechnology, Santa Cruz, CA), mouse anti-neuronal nuclear protein (NeuN; 1:200; Chemicon), goat anti-IGF-I Abs (1:20; R&D Systems), goat anti-TNF-α Abs (1:100; R&D Systems), rabbit anti-CD3 polyclonal Abs (1:100; DakoCytomation, CA). Secondary antibodies: FITC-conjugated donkey anti-goat, Cy-3-conjugated donkey anti-mouse, and Cy-3- or Cy-5-conjugated donkey anti-rat, biotin-conjugated anti-hamster antibody and Cy-3- or Cy-5-conjugated streptavidin antibody (all from Jackson ImmunoResearch).

Cover slips from co-cultures of NPCs and mouse microglia were washed with PBS, fixed as described above, treated with a permeabilization/blocking solution containing 10% FCS, 2% bovine serum albumin, 1% glycine, and 0.1% Triton X-100 (Sigma-Aldrich, Rehovot), and stained with a combination of mouse anti-β-tubulin (anti-βIII-T) isoform C-terminus antibodies (1:500; Chemicon, Temecula, CA), rat anti-CD11b (MAC1; 1:50; BD–Pharmingen, Franklin Lakes, NJ) and hamster anti-CD11c (1:100; eBioscience, San Diego, CA). To capture the microglia FITC- or Cy3-conjugated *Bandeiraea simplicifolia* isolectin B4 (IB-4; 1:50; Sigma-Aldrich, Rehovot) was used. To detect expression of human Aβ anti-Aβ (human amino-acid residues 1–17) (mouse, clone 6E10; Chemicon) was used.

For BrdU staining, sections were washed with PBS and incubated in 2N HCl at 37°C for 30 min. Sections were blocked for 1 h with blocking solution [PBS containing 20% normal horse serum and 0.1% Triton X-100, or PBS containing mouse immunoglobulin blocking reagent obtained from Vector Laboratories (Burlingame, CA)].

For immunohistochemistry, tissue sections were treated with a permeabilization/blocking solution containing 10% FCS, 2% bovine serum albumin, 1% glycine, and 0.05% Triton X-100 (Sigma-Aldrich, St. Louis). Tissue sections were stained overnight at 4°C with specified combinations of the following primary antibodies: rat anti-BrdU (1:200; Oxford Biotechnology, Kidlington, Oxfordshire, UK), goat anti-doublecortin (anti-DCX) (1:400; Santa Cruz Biotechnology, Santa Cruz, CA), and mouse anti-neuronal nuclear protein (anti-NeuN) (1:200; Chemicon). Secondary antibodies were FITC-conjugated donkey anti-goat, Cy-3-conjugated donkey anti-mouse, and Cy-3- or Cy-5-conjugated donkey anti-rat (1:200; Jackson

ImmunoResearch, West Grove, PA). CD11b (MAC1; 1:50; BD–Pharmingen) or FITC-conjugated IB-4 was used for labeling of microglia. Anti-MHC-II Abs (rat, clone IBL-5/22; 1:50) was used to detect expression of cell-surface MHC-II proteins. To detect expression of CD11c hamster anti-CD11c (1:100; eBioscience, San Diego, CA) was used. Anti-Aβ (human amino-acid residues 1–17) (mouse, clone 6E10; Chemicon) was used to detect expression of human Aβ. Expression of IGF-I was detected by goat anti-IGF-I Abs (1:20; R&D Systems). Expression of TNF-α was detected by goat anti-TNF-α Abs (1:100; R&D Systems). T cells were detected with anti-CD3 polyclonal Abs (rabbit, 1:100; DakoCytomation, CA). Propidium iodide (1 μg/ml; Molecular Probes, Invitrogen, Carlsbad, CA), was used for nuclear staining.

Control sections (not treated with primary antibody) were used to distinguish specific staining from staining of nonspecific antibodies or autofluorescent components. Sections were then washed with PBS and cover-slipped in polyvinyl alcohol with diazabicyclo-octane as anti-fading agent.

(v) Transgenic mice. Nineteen adult double-transgenic $APP_{K595N, M596L}$ + $PS1_{\Delta E9}$ mice of the B6C3-Tg (APPswe, PSEN1dE9) 85Dbo/J strain (Borchelt et al., 1997) were purchased from The Jackson Laboratory (Bar Harbor, ME) and were bred and maintained in the Animal Breeding Center of The Weizmann Institute of Science. All animals were handled according to the regulations formulated by the Weizmann Institute's Animal Care and Use Committee, and all experiments and procedures were approved by the Weizmann Institute's Animal Care and Use Committee.

(vi) Genotyping. All mice used in this experiment were genotyped for the presence of the transgenes by PCR as previously described (Jankowsky et al., 2004).

(vii) Reagents. Recombinant mouse IFN-γ and IL-4 were obtained from R&D Systems (Minneapolis, MN). β-amyloid peptide [fragment 1–40 ($A\beta_{1-40}$)] was purchased from Sigma-Aldrich, St. Louis, MO. The Aβ peptide was dissolved in endotoxin-free water, and Aβ aggregates were formed by incubation of Aβ, as described (Butovsky et al., 2005).

(viii) Copolymer-1 vaccination. Each mouse was subcutaneously injected five times with a total of 100 μg of high-molecular-weight (TV-5010 DS, from batch no. 486220205; Teva Pharmaceutical Industries, Petach Tikva, Israel) emulsified in 200 μl PBS, from experimental day 0 until day 24, twice during the first week and once a week thereafter.

(ix) Behavioral testing. Spatial learning/memory was assessed by performance on a hippocampus-dependent visuo-spatial learning task in the Morris water maze (MWM) and carried out as described (Lichtenwalner et al., 2001).

(x) Administration of 5-bromo-2'-deoxyuridine and tissue preparation. The cell-proliferation marker 5-bromo-2'-deoxyuridine (BrdU) was dissolved by sonication in phosphate-buffered saline (PBS) and injected intraperitoneally (i.p.) into each mouse (50 mg/kg body weight; 1.25 mg BrdU in 200 μl PBS). Starting from experimental day 22 after the first Cop-1 vaccination, BrdU was injected i.p. twice daily, every 12 h for 2.5 days, to label proliferating cells. Three weeks after the first BrdU injection the mice were deeply anesthetized and perfused transcardially, first with PBS and then with 4% paraformaldehyde. The whole brain was removed, postfixed overnight, and then equilibrated in phosphate-buffered 30% sucrose. Free-floating 30-μm sections were collected on a freezing microtome (Leica SM2000R) and stored at 4°C prior to immunohistochemistry.

(xi) Co-culturing of neural progenitor cells and microglia. Cultures of treated or untreated microglia were washed twice with fresh NPC-differentiation medium (same as the culture medium for NPCs but without growth factors except for 0.02 mg/ml insulin and with 2.5% FCS) to remove all traces of the tested reagents, then incubated on ice for 15 min and shaken at 350 rpm for 20 min at room temperature. Microglia were removed from the flasks and immediately co-cultured (5×10^4

cells/well) with NPCs (5×10^4 cells/well) for 10 days on cover slips coated with Matrigel™ (BD Biosciences) in 24-well plates, in the presence of NPC-differentiation medium. The cultures were then fixed with 2.5% paraformaldehyde in PBS for 30 min at room temperature and stained for neuronal and glial markers.

(xii) Quantification and stereological counting procedure. A Zeiss LSM 510 confocal laser scanning microscope (×40 magnification) was used for microscopic analysis. For experiments *in vitro* fields of 0.053 mm^2 (n = 8–16 from at least two different cover slips) were scanned for each experimental group. For each marker, 500–1000 cells were sampled. Cells co-expressing GFP and βIII-T were counted.

For *in-vivo* experiments, the numbers of Aβ plaques and $CD11b^+$ microglia in the hippocampus were counted at 300-μm intervals in 6–8 coronal sections (30 μm) from each mouse. Neurogenesis in the dentate gyrus was evaluated by counting of pre-mature neurons (DCX^+), proliferating cells ($BrdU^+$), and newly formed mature neurons ($BrdU^+/NeuN^+$) in six coronal sections (370 μm apart) per mouse brain. To obtain an estimate of the total number of labeled cells per dentate gyrus, the total number of cells counted in the selected coronal sections from each brain was multiplied by the volume index (the ratio between the volume of the dentate gyrus and the total combined volume of the selected sections). Specificity of BrdU+/NeuN+ co-expression was assayed using the confocal microscope (LSM 510) in optical sections at 1-μm intervals. Quantification of $CD3^+$, $CD11b^+$ and $CD11c^+$ cells were analyzed from 30–50 Aβ-plaques of each mouse tested in this study. Cell counts, numbers of Aβ plaques, plaque areas, and intensity of NeuN staining per unit area in the dentate gyrus were evaluated automatically using Image-Pro Plus 4.5 software (Media Cybernetics, Carlsbad, CA).

(xiii) Statistical analysis. MWM behavior scores were analyzed using 3-way ANOVA. Treatment group and trial block were used as sources of variation to evaluate the significance of differences between mean scores during acquisition trial blocks in the MWM. When the P-value obtained was significant, a pairwise Fisher's least-significant-difference multiple comparison test was run to determine which groups were significantly different.

The *in-vitro* results were analyzed by two-tailed unpaired Student's *t*-test and by the Tukey–Kramer multiple comparisons test (ANOVA) and are expressed as means ± SEM. Results *in vivo* were analyzed by two-tailed unpaired Student's *t*-test or 1-way ANOVA and are expressed as means ± SEM.

Example 1. Aggregated β-amyloid induces microglia to express a phenotype that blocks neurogenesis, and the blocking is counteracted by IL-4.

Previous in vitro findings from our laboratory have suggested that the microglia found in association with inflammatory and neurodegenerative diseases (e.g. microglia activated by LPS or by aggregated $A\beta_{(1-40)}$) have an impaired ability to present antigen, whereas IL-4-activated microglia, shown to be associated with neural tissue survival, express MHC-II, produce IGF-I, and decrease TNF-α expression (Butovsky et al., 2005). Here we first examined whether Aβ-activated microglia block neurogenesis, and if so, whether T cell-derived cytokines can counteract the inhibitory effect. To this end we co-cultured green fluorescent protein (GFP)-expressing neural stem/progenitor cells (NPCs) with microglia that had been pre-incubated for 48 h in their optimal growth medium (Butovsky et al., 2005) in the presence or absence of the aggregated Aβ peptide 1–40 ($A\beta_{(1-40)}$; 5 μM) and subsequently treated for an additional 48 h with IFN-γ (10 ng/ml) or IL-4 (10 ng/ml) or IL-4 together with IFN-γ (10 ng/ml). The choice of $A\beta_{(1-40)}$ rather than $A\beta_{(1-42)}$ and its concentration was based on our previous demonstration that this compound induces cytotoxic activity in microglia (Butovsky et al., 2005). Growth media and cytokine residues were then washed off the co-cultured microglia, and each of the treated microglial preparations was freshly co-cultured with dissociated adult subventricular zone-derived NPC spheres (Butovsky et al., 2006a) on coverslips coated with Matrigel™ in the presence of differentiation medium (Butovsky et al., 2006a) (**Fig. 1A**). Expression of GFP by NPCs confirmed that any differentiating neurons seen in the cultures were derived from the NPCs rather than from contamination of the primary microglial culture. After 10 days, we could discern a few GFP-positive NPCs expressing the neuronal marker βIII-T in microglia-free cultures (control). In co-cultures of NPCs with microglia previously activated by incubation with IFN-γ (10 ng/ml; $MG_{(IFN\text{-}\gamma)}$) a dramatic increase in numbers of $GFP^+/\beta III\text{-}T^+$ cells was seen. On the contrary, microglia activated by aggregated $A\beta_{(1-40)}$ ($MG_{(A\beta1\text{-}40)}$) blocked neurogenesis and decreased the number of NPCs. The addition of IFN-γ to Aβ-activated microglia ($MG_{(A\beta1\text{-}40/IFN\text{-}\gamma)}$), failed to reverse their negative effect on neurogenesis. In contrast, the addition of IL-4 (10 ng/ml) to microglia pretreated with aggregated $A\beta_{(1-40)}$ ($MG_{(A\beta1\text{-}40/IL\text{-}4)}$) partially counteracted the adverse effect of the aggregated Aβ on NPCs survival and differentiation, with the result that these microglia were able to induce NPCs to differentiate into neurons. However, when IFN-γ was added in combination with IL-4 ($MG_{(A\beta1\text{-}40/IFN\text{-}\gamma+IL\text{-}4)}$), their effect in counteracting the negative activity of the Aβ-activated microglia on NPC survival and differentiation was stronger than the effect of IL-4 alone (**Fig. 1B**). We verified that in all cases the $\beta III\text{-}T^+$ cells also expressed GFP (**Fig. 1C**). This finding is particularly interesting in view of our earlier demonstration that the order in which threatening stimuli are presented to the microglia critically affects the ability of these cells to withstand them (Butovsky et al., 2005). The quantitative analysis presented in **Fig. 1D** summarizes the data shown in **Fig. 1B**, and in addition shows that differentiation in the presence of untreated microglia occurred only to a small extent. Notably, no $\beta III\text{-}T^+$ cells were seen in microglia cultured without NPCs (Butovsky et al., 2006a).

Example 2. T-cell-based vaccination with copolymer-1 modulates immune activity of microglia, eliminates β-amyloid plaque formation, and induces neurogenesis.

The above findings prompted us to examine whether a T cell-based vaccination would alter the default microglial phenotype in Alzheimer's disease and hence lead to plaque removal and neurogenesis. The antigen we chose for the vaccination was Cop-1 (Teitelbaum et al., 1996), shown by us to be weakly cross-reactive with a wide-range of CNS autoantigens and, depending on the regimen, to be neuroprotective under conditions of both acute and chronic neurodegeneration (Kipnis et al., 2000; Schori et al., 2001; Angelov et al., 2003). We examined the effect of Cop-1 in Tg-AD mice, suffering from learning/memory impairment and an accumulation of aggregated Aβ plaques deposited mainly in the cortex and the hippocampus, both characteristic features of early-onset familial Alzheimer's disease (Borchelt et al., 1997). The regimen for Cop-1 administration was similar to that used to evoke neuroprotection in a model of chronic elevation of intraocular pressure (Bakalash et al., 2005).

We verified the presence of both transgenes in each mouse by PCR amplification of genomic DNA. Tg-AD mice aged approximately 8 months were then vaccinated subcutaneously with Cop-1 (n = 6) twice during the first week and once a week thereafter. Age-matched untreated Tg-AD mice (n = 7) and non-Tg littermates (n = 6) served, respectively, as untreated-Tg and wild-type controls. Seven weeks after the first Cop-1 injection all the mice were euthanized and analyzed. Staining of brain cryosections from Tg-AD mice with antibodies specific to human Aβ disclosed numerous plaques in the untreated-Tg-AD mice but very few in the Tg-AD mice vaccinated with Cop-1 (**Fig. 2*A***). No plaques were seen in their respective non-Tg littermates (**Fig. 2*A***).

The above results, coupled with the *in-vitro* findings, prompted us to look for changes in microglial features in the vaccinated Tg-AD mice. Plaques in the untreated-Tg-AD mice were found to be associated with the abundant appearance of CD11b$^+$ microglia (**Fig. 2*A*** and **Fig 2*B***) expressing TNF-α (**Fig. 2*C***). Fewer CD11b$^+$ microglia were detectable in the Cop-1-vaccinated Tg-AD mice (**Fig. 2*A***). It is important to note that the CD11b$^+$ microglia in the untreated-Tg-AD mice showed relatively few ramified processes **Fig. 2C**). Staining with anti-MHC-II antibodies disclosed that in the Cop-1-vaccinated Tg-AD mice most of the microglia adjacent to residual Aβ plaques expressed MHC-II, and hardly any of them expressed TNF-α (**Fig. 2*D***), whereas in the untreated-Tg-AD mice hardly any microglia expressed MHC-II (**Fig. 2*E***), suggesting that their ability to function as APCs is limited. All of the MHC-II$^+$ cells were co-labeled with IB-4 (data not shown), verifying their identification as microglia. The dendritic-like morphology (**Fig. 2*D***) of the MHC-II$^+$ microglia seen in the Cop-1-vaccinated Tg-AD mice encouraged us to examine whether they express the characteristic marker of dendritic cells, namely CD11c. CD11c$^+$ microglia in untreated Tg-AD mice were only rarely found in association with Aβ$^+$ plaques, whereas any residual Aβ-stained plaques seen in the Cop-1-vaccinated mice were surrounded by MHC-II$^+$/CD11c$^+$ microglia (**Fig. 2E**). Notably, these CD11c$^+$ microglia were also positively stained for CD11b **(Fig. 3A)**; in addition, they were loaded with Aβ, indicative of their engulfment of this peptide (**Fig. 2E**). Quantitative analysis revealed that the number of CD11b$^+$ cells associated with Aβ-plaque significantly decreased in the Cop-1-vaccinated Tg-AD mice (**Fig. 3B**), and that as a result of the vaccination 87 % of the CD11b$^+$ became CD11b$^+$/CD11c$^+$ cells relative to 25 % in the untreated Tg-AD mice (**Fig. 3C**).

In view of our recent finding that MHC-II$^+$ microglia (which are activated by IL-4) abundantly express IGF-I (Butovsky et al., 2005; Butovsky et al., 2006a;b), we examined IGF-I expression in the vaccinated Tg-AD mice. MHC-II$^+$ microglia in these mice were indeed found to express IGF-I (**Fig. 2F**). Staining for the presence of T cells, identified by anti-CD3 antibodies, revealed that unlike in the untreated-Tg-AD mice, in the Cop-1-vaccinated Tg-AD mice there were numerous T cells associated with Aβ-plaques **(Fig. 2G)**. Moreover, most of the T cells in the Cop-1 vaccinated Tg-AD mice were found to be located close to MHC-II$^+$ microglia. Any Aβ-immunoreactivity detected in those mice appeared to be associated with the MHC-II$^+$ microglia, suggesting the occurrence of an immune synapse between these microglia and CD3$^+$ T cells (**Fig. 2H**). Quantitative analysis confirmed the presence of significantly fewer plaques in the Cop-1-vaccinated Tg-AD mice than in the untreated-Tg-AD mice (**Fig. 2I**), and showed that the area occupied by the plaques was significantly smaller in the vaccinated Tg-AD mice than in their age-matched untreated counterparts (**Fig. 2J**). In addition, significantly fewer CD11b$^+$ microglia (**Fig. 2K**) and significantly more T cells associated with Aβ-plaque were observed in the Cop-1-vaccinated Tg-AD mice than in the corresponding groups of untreated-Tg-AD mice (**Fig. 2L**).

On the basis of our previous findings, we suspected that the switch from a CD11b$^+$/CD11c$^-$/IGF-1$^-$ to a CD11b$^+$/CD11c$^+$/IGF-I$^+$ microglial phenotype in the Cop-1 vaccinated Tg-AD mice might be attributable to IL-4. We examined this possibility *in vitro*. Staining of 5-day microglia cultures with the CD11c marker showed that CD11c was hardly expressed at all by untreated, but was abundantly expressed by microglia activated by IL-4 (**Fig. 4A**). Moreover, IL-4, even if only added 3 days after the microglia were exposed to Aβ, was able to induce them to express CD11c (**Fig. 4B**). Differential activation of the microglia was also reflected in morphological differences: microglia activated by Aβ exhibited amoeboid morphology, whereas the rounded shape of the CD11c$^+$ microglia was reminiscent of dendritic cells (**Fig. 4B**). Most importantly, the amoeboid morphology of the Aβ-stained microglia was reversible on addition of IL-4, when they again took on the morphological appearance of dendritic-like cells (**Fig. 4B**). The various treatments applied to the microglia did not affect their expression of CD11b, suggesting that they did not lose their CD11b characteristics when they took on the expression of CD11c (**Fig. 4B**). Quantitative analysis of CD11c expression, assessed by the number of CD11c$^+$ cells and the intensity of their staining as a function of time in culture, revealed that soon after seeding (day 0) untreated microglia expressed low levels of CD11c, which gradually disappeared (**Fig. 4C**). In contrast, the expression of CD11c induced by IL-4 was not transient. Quantification of the ability of IL-4 to induce CD11c expression even after the microglia were pretreated with Aβ is shown in **Fig. 4D**.

The correlation between the phenotype that was found to be induced by the Cop-1 vaccination and the IL-4 effect on microglia *in vitro* prompted us to examine the ability of IL-4-activated microglia to phagocytize aggregated $A\beta_{(1-40)}$. Quantitative comparison (by intracellular staining) of immunoreactive Aβ engulfed by IL-4-treated and untreated microglia indicated that IL-4 did not interfere with the ability of microglia to engulf Aβ (**Fig. 5**).

The observed effects of IL-4 on the expression of CD11c, MHC-II, and TNF-α prompted us to examine whether the Cop-1-vaccinated Tg-AD mice would show increased neurogenesis *in vivo*. Three weeks before tissue excision all mice had been injected with the proliferating-cell marker BrdU, making it possible to detect new neurons. Quantitative analysis of additional sections from the same areas of the hippocampal dentate gyrus disclosed significantly more $BrdU^+$ cells in the Cop-1-vaccinated Tg-AD mice (**Fig. 6A**) than in their untreated-Tg counterparts. In addition, compared to the numbers of newly formed mature neurons ($BrdU^+/NeuN^+$) in their respective non-Tg littermates the numbers were significantly lower in the untreated Tg-AD group, but were similar in the Cop-1-vaccinated Tg-AD group; indicating that the neurogenesis capacity had been at least partially restored by the Cop-1 vaccination (**Fig. 6B**). Analysis of corresponding sections for DCX, a useful marker for analyzing the absolute number of newly generated pre-mature neurons in the adult dentate gyrus (Rao et al., 2004), disclosed that relative to the non-Tg littermates there were significantly fewer DCX^+ cells in the dentate gyri of untreated-Tg-AD mice, and slightly but significantly more in the dentate gyri of Tg-AD mice vaccinated with Cop-1 (**Fig. 6C**). Confocal micrographs illustrate the differences in the numbers of $BrdU^+/NeuN^+$ cells, and in the numbers of DCX^+ cells and their dendritic processes, between non-Tg littermates, untreated-Tg-AD mice, and Cop-1-vaccinated Tg-AD mice (**Fig. 6D**). The results showed that neurogenesis was indeed significantly more abundant in the Cop-1-treated Tg-AD mice than in the untreated-Tg-AD mice. Interestingly, however, in both untreated and Cop-1-vaccinated Tg-AD mice the processes of the DCX-stained neurons in the subgranular zone of the dentate gyrus were short, except in those Cop-1-vaccinated Tg-AD mice in which the DCX^+ cells were located adjacent to MHC-II^+ microglia (**Fig. 6E**).

Discussion

We showed here that vaccination of Tg-AD mice with Cop-1 reduced plaque formation and attenuated cognitive decline. Labeling of activated microglia with anti-CD11b antibodies disclosed that staining was heavy in the untreated-Tg-AD mice and significantly less intense in the age-matched Cop-1-vaccinated Tg-AD mice. The decrease in numbers of $CD11b^+$ microglia in Cop-1-vaccinated mice could be an outcome of a reduction in Aβ-plaques, whose deposition in the brain had led to the microglial activation in the first place.

The role of microglia in Alzheimer's disease and other neurodegenerative diseases has been intensively investigated over the last few years, with apparently conflicting results (Streit, 2004). The detection of some $CD11b^+$ microglia in aged wild-type mice that are healthy is in line with the reported age-related increase in activated microglia in the normal human brain (Streit, 2004). It is possible that such microglia are the ones that contribute both to age-related cognitive loss and to impaired neurogenesis (Monje et al., 2003). CD11b were found also in patients with Alzheimer's disease (Akiyama & McGeer, 1990). Although these microglia are phagocytic (Frenkel et al., 2005), they are apparently not efficient enough to fight off the Alzheimer symptoms. In contrast, and in line with our present study, microglia derived from the bone marrow of matched wild-type mice can effectively remove plaques (Simard et al., 2006). These microglia express higher protein levels than are required for antigen presentation and might therefore be more effective phagocytes than the resident microglia. On the basis of our present results, we suggest that the microglia that are needed to support brain maintenance and fight Alzheimer's disease are dendritic-like cells ($CD11b^+/CD11c^+$). The $CD11c^+$ microglia might engage in a dialog with T cells that can help to fight off adverse conditions by promoting the buffering of excessive Aβ and supporting both neuronal survival (Butovsky et al., 2005) and neural renewal (Butovsky et al., 2006b). In view of our earlier finding that IL-4, but not IFN-γ, can alter the phenotype of Aβ-committed microglia (Butovsky et al., 2005), it seems likely that the MHC-II$^+$ microglia found adjacent to Aβ plaques in the present study were activated by IL-4. This likelihood is further supported by two of the *in-vitro* findings of the present study: first, that the Aβ-induced blockage of neurogenesis was partially counteracted by IL-4, either alone or in combination with IFN-γ, but not by IFN-γ alone; and secondly, that the MHC-II$^+$/$CD11c^+$ microglia which were seen in close proximity to the residual Aβ plaques in the Cop-1-vaccinated Tg-AD mice also expressed IGF-I. Taken together, these findings reinforce the contention that the terms 'beneficial' and 'harmful' cannot be applied in a generalized way to microglial activity (Schwartz et al., 2006).

IL-4 has often been described as an anti-inflammatory cytokine (Chao et al., 1993). Our results strongly argue against this perception and suggest instead that IL-4 activates microglia to adopt a phenotype that seems to acquire a different morphology and a different activity from those of the innately activated microglia or of the activated microglia commonly seen in Alzheimer's disease or in MS. In the latter disease, unlike in Alzheimer's disease, the microglia appear to be overwhelmed by an onslaught of adaptive immunity (Butovsky et al., 2006a). Interestingly, it seems that IL-4 is capable of restoring a favorable activated phenotype even after the microglia have already exhibited phenotypic characteristics of aggregated Aβ ((Butovsky et al., 2005), and the present study), or been overwhelmed by IFN-γ (Butovsky et al., 2006a). Another interesting finding is that LPS and Aβ exhibit similar patterns of MAPK activation in microglia (Pyo et al., 1998). IL-4 can attenuate a MAPK pathway activated by LPS, an effect evidently associated with serine/threonine phosphatase activity (Iribarren et al., 2003). The latter phenomenon might indeed serve as a molecular mechanism underlying the present finding that IL-4 attenuates the detrimental effect of Aβ-activated microglia. Accordingly, we suggest that the activity of IL-4 should not be regarded as anti-inflammatory, but as immunomodulatory.

Based on our *in-vitro* findings in connection with the effect of IL-4 on microglial phenotype, and the ability of the regimen chosen in the present study to evoke a T-cell response with an IL-4 bias, we suggest that the observed beneficial effect of Cop-1 vaccination on the Tg-AD mice in this study was a result of the evoked T-cell effect on their microglial phenotype.

Example 3. Copolymer-1 vaccination counteracts cognitive decline in Alzheimer's disease.

Two weeks before the end of the experiment, all mice were tested in a Morris water maze (MWM) for cognitive activity, as reflected by their performance of a hippocampus-dependent spatial learning/memory task. The MWM performance of the untreated-Tg-AD mice was significantly worse, on average, than that of their age-matched non-Tg littermates (**Figs. 7A-7B**). However, the performance of Cop-1-vaccinated Tg-AD mice was superior to that of the untreated-Tg-AD mice and did not differ significantly from that of the non-Tg-AD mice, suggesting that the Cop-1 vaccination had prevented further cognitive loss. Differences in cognitive performance were manifested in both the acquisition (**Fig. 7A**) and the reversal tasks (**Fig. 7B**).

The vaccinated mice in this study demonstrated attenuated cognitive loss (tested in a Morris water maze) and increased neurogenesis. These two aspects of hippocampal plasticity are apparently related to the presence of IGF-I and cognitive activity and cell renewal (Butovsky et al., 2006a;b). Reported observations in Tg-AD mice housed in an enriched environment also support a link between mechanisms associated with neurogenesis (Ziv et al., 2006) and with plaque reduction (Lazarov et al., 2005).

Because aggregated Aβ evidently interferes with the ability of microglia to engage in dialog with T cells, its presence in the brain can be expected to cause loss of cognitive ability and impairment of neurogenesis. Homing of CNS-autoreactive T cells to the site of disease or damage in such cases is critical, but will be effective only if those T cells can counterbalance the destructive activity of the aggregated Aβ. As shown here, IFN-γ by itself is impotent against the activity of microglia that are already committed to an aggregated Aβ phenotype, but is effective when added together with IL-4. Thus, the results of this study strongly suggest that the occurrence of neurogenesis in the adult hippocampus depends on well controlled local immune activity associated with microglial production of growth factors such as IGF-I and BDNF (Ziv et al., 2006). In line with this notion is the reported finding that neurogenesis is impaired in animals treated with LPS (Monje et al., 2003), shown to impair microglial production of IGF-I and induce microglial secretion of TNF-α (Butovsky et al., 2005).

Example 4. IFN-γ-activated myeloid cells and their uses for promoting tissue repair, detection of and delivery of drugs to damaged tissue.

We have shown that when IFN-γ was added in combination with IL-4 their effect in counteracting the negative activity of Aβ-activated microglia on NPC survival and differentiation was stronger than the effect of IL-4 alone (**Fig. 1B**). However, our data demonstrates that IFN-γ alone, similarly to IL-4 but transiently, induces CD11c if the cells are treated with IFN-γ in a narrow concentration range of up to 20 ng/ml, induces neuroprotection (Butovsky et al., 2005) and is able to support neurogenesis from neural stem/progenitor cells (Butovsky et al., 2006b). CD11c is upregulated and reach the peak at 2-3 days after IFN-γ activation in microglia (**Figs. 8A-8D**) or bone-marrow-derived myeloid cells (data not shown). Thus, upregulation of CD11c on bone marrow derived myeloid cells by IFN-γ will allow the cells to reach injured sites and induce beneficial effect.

Example 5. Weekly vaccination with Copaxone as a potential therapy for dry Age-related Macular Degeneration

The results of the previous examples showed that vaccination of Tg-AD mice with Cop-1 in a regimen previously found to lead to neuroprotection resulted in a microglial phenotype switch from $CD11b^{+}$ to $CD11b^{+}/CD11c^{+}$, and that this was correlated with plaque removal, neurogenesis, and attenuated cognitive loss. The beneficial effect was attributed to a phenotype switch of the infiltrating microglia as well as to recruitment of blood-borne monocytes.

Based on these findings and on the many features common between Alzheimer's disease and AMD, we hypothesized that in AMD, similar to Alzheimer's disease, weekly Cop-1 treatment would likely lead to clearing of drusen and this, subsequently, may restrain the progression of dry to wet AMD. It should be emphasized that Cop-1 given in different regimens results in different therapies; daily treatment has no beneficial effects in paradigms of neurodegenerative diseases, but is effective in suppressing inflammatory disease in patients like multiple sclerosis. Therefore, we decided to embark on a clinical trial with AMD patients in a protocol of weekly administration.

The natural fate of drusen in our population of patients was examined by analysis of fundus photographs of unenrolled and untreated dry AMD patients. These patients comprised an observational group.

The effect of Cop-1 (Copaxone®, Teva) on drusen was examined during a prospective, pilot, randomized, double-masked, placebo-controlled, comparative trial. Patients over 50 years of age with intermediate dry AMD in both eyes were enrolled.

Enrolled patients were randomly (ratio 2 to 1) treated either with subcutaneous injections of Copaxone (20 mg) or placebo injections on a weekly basis for a period of 12 weeks. Complete eye examination, along with fundus photography, was performed at baseline, 6- and 12-week visits. The primary outcome was the change in total drusen area and it was calculated using Image-Pro software. Analysis of patients from the observational group (17 eyes) showed an increase of 25.2% in total drusen area.

At 12 week, 8 eyes treated with Copaxone showed a reduction in total drusen area from baseline of 53.6% (range, 5-89%). Two eyes receiving placebo injections demonstrated reduction of 0.6% in total drusen area.

This study thus shows a potential beneficial effect for Copaxone for treating AMD. Due to the limited numbers of the enrolled patients, no statististical significant conclusion can be drawn. Yet, the results justify and encourage to extend and continue this study for fighting off drusens. In light of the underlying mechanism of weekly treatment with Copaxone in animal models of neurodegenerative diseases, its effect on AMD might be beyond the drusens' elimination in protecting neurons and promoting repair.

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CLAIM

1. A method for treatment of age-related macular degeneration, which comprises causing T cells that produce IL-4 to accumulate in the eye of an individual in need, thereby halting or delaying progress of the macular degeneration.

2. The method according to claim 1, wherein said accumulation of T cells in the eye is caused by administering to said individual an agent selected from the group consisting of:

(i) Copolymer-1, a Copolymer-1-related-peptide, or a Copolymer 1-related polypeptide;

(ii) IL-4;

(iii) dendritic cells, monocytes, bone marrow-derived myeloid cells or peripheral blood mononuclear cells activated by IL-4;

(iv) genetically engineered cells that produce IL-4;

(v) bone marrow-derived myeloid cells or peripheral blood-derived myeloid cells activated with IL-13 or with up to 20 ng/ml IFN-γ;

(vi) a pathogenic self-antigen associated with a T-cell-mediated specific autoimmune disease of the eye;

(vii) a peptide which sequence is comprised within the sequence of said pathogenic self-antigen of (vi) or a peptide obtained by modification of said peptide, which modification consists in the replacement of one or more amino acid residues of the peptide by different amino acid residues (hereinafter "modified peptide"), said modified peptide still being capable of recognizing the T-cell receptor recognized by the parent peptide but with less affinity;

(viii) a nucleotide sequence encoding a pathogenic self-antigen of (vi) or a peptide or a modified peptide of (vii);

(ix) T cells activated by an agent of (i), (vi) or (vii); and

(x) any combination of (i) – (ix).

3. The method according to claim 2, wherein said agent is Copolymer 1, a Copolymer 1-related peptide or a Copolymer 1-related polypeptide.

4. The method according to claim 3, wherein said agent is Copolymer 1.

5. The method according to claim 3, wherein said Copolymer 1-related polypeptide is a random copolymer comprising one amino acid residue selected from each of at least three of the following groups:

(a) lysine and arginine;
(b) glutamic acid and aspartic acid;
(c) alanine and glycine; and
(d) tyrosine and tryptophan.

6. The method according to claim 5, wherein said random copolymer consists of four different amino acid residues, each from a different one of the groups (a) to (d).

7. The method according to claim 6, wherein said four different amino acid residues are alanine, glutamic acid, lysine and tyrosine.

8. The method according to claim 5, wherein said random copolymer consists of three different amino acid residues, each from a different one of three groups (a) to (d).

9 The method according to claim 8, wherein said random copolymer is a terpolymer selected from the group consisting of YAK, YEK, KEA and YEA.

10 The method according to claim 3, wherein said agent is a Copolymer 1-related peptide selected from the peptides represented by SEQ ID Nos. 1-32.

11. The method according to claim 2, wherein said agent is T cells activated by Copolymer 1.

12. The method according to claim 2, wherein said agent is IL-4.

13. The method according to claim 12, wherein IL-4 is administered nasally.

14. The method according to claim 2, wherein said agent is IL-4 activated dendritic cells, monocytes, bone marrow-derived myeloid cells or peripheral blood mononuclear cells autologous or from a matched donor.

15. The method according to claim 2, wherein said agent is bone marrow-derived myeloid cells or peripheral blood-derived myeloid cells activated with IL-13 or with up to 20 ng/ml IFN-γ.

16. The method according to claim 2, wherein said pathogenic self-antigen associated with a T-cell-mediated specific autoimmune disease of the eye is a pathogenic uveitogenic antigen selected from the group consisting of human interphotoreceptor retinoid-binding protein (IRBP) (SEQ ID NO: 33), bovine IRBP (SEQ ID NO: 34), human S-antigen (S-Ag) (SEQ ID NO: 35), bovine S-Ag (SEQ ID NO: 36) and human rhodopsin (SEQ ID NO: 37).

17. The method according to claim 16, wherein said agent is selected from the group consisting of:

(a) a peptide which sequence is comprised within the sequence of bovine IRBP (SEQ ID NO: 34);

(b) a peptide obtained by modification of the peptide of (a), which modification consists in the replacement of one or more amino acid residues of the peptide by different amino acid residues, said modified peptide still being capable of recognizing the T-cell receptor recognized by the parent peptide but with less affinity (hereinafter “modified peptide”);

(c) a nucleotide sequence encoding human IRBP (SEQ ID NO: 33) or bovine IRPB, a peptide of (a), or a modified peptide of (b) ; and

(d) T cells activated by an agent selected from the group consisting of human or bovine IRPB, a peptide of (a), and a modified peptide of (b).

18. The method according to claim 17, wherein said peptide (b) is selected from the group consisting of the peptides:

ADGSSWEGVGVVPDV (SEQ ID NO: 38);
PTARSVGAADGSSWEGVGVVPDV (SEQ ID NO: 39); and
HVDDTDLYLTIPTARSVGAADGS (SEQ ID NO: 40).

19. The method according to claim 16, wherein said agent is selected from the group consisting of:

(a) a peptide which sequence is comprised within the sequence of bovine S-Ag (SEQ ID NO: 36);

(b) a peptide obtained by modification of the peptide of (a), which modification consists in the replacement of one or more amino acid residues of the peptide by different amino acid residues, said modified peptide still being capable of recognizing the T-cell receptor recognized by the parent peptide but with less affinity (hereinafter "modified peptide");

(c) a nucleotide sequence encoding human S-Ag (SEQ ID NO: 35) or bovine S-Ag, a peptide of (a), or a modified peptide of (b) ; and

(d) T cells activated by an agent selected from the group consisting of human or bovine S-Ag, a peptide of (a), and a modified peptide of (b).

20. The method according to claim 19, wherein said peptide (b) is selected from the group consisting of the peptides:

TSSEVATE (SEQ ID NO:41);
DTNLASST (SEQ ID NO:42);
DTNLASSTIIKEGIDKTV (SEQ ID NO:43);
VPLLANNRERRGIALDGKIKHE (SEQ ID NO:44);
TSSEVATEVPFRLMHPQPED (SEQ ID NO:45);
SLTKTLTLVPLLANNRERRG (SEQ ID NO:46);
SLTRTLTLLPLLANNRERAG (SEQ ID NO:47);
KEGIDKTVMGILVSYQIKVKL (SEQ ID NO:48); and
KEGIDRTVLGILVSYQIKVKL (SEQ ID NO:49).

21. The method according to claim 19, wherein said modified peptide (c) is selected from the group consisting of the peptides:

TSSEAATE (SEQ ID NO:50); and

DTALASST (SEQ ID NO:51).

22. Use of an agent that causes T cells that produce IL-4 to accumulate in the eye for the preparation of a medicament for treatment of age-related macular degeneration.

23. The use according to claim 22, wherein said agent is selected from:

(i) Copolymer-1, a Copolymer-1-related-peptide, or a Copolymer 1-related polypeptide;

(ii) IL-4;

(iii) dendritic cells, monocytes, bone marrow-derived myeloid cells or peripheral blood mononuclear cells activated by IL-4;

(iv) genetically engineered cells that produce IL-4;

(v) bone marrow-derived myeloid cells or peripheral blood-derived myeloid cells activated with IL-13 or with up to 20 ng/ml IFN-γ:

(vi) a pathogenic self-antigen associated with a T-cell-mediated specific autoimmune disease of the eye;

(vii) a peptide which sequence is comprised within the sequence of said pathogenic self-antigen of (vi) or a peptide obtained by modification of said peptide, which modification consists in the replacement of one or more amino acid residues of the peptide by different amino acid residues (hereinafter "modified peptide"), said modified peptide still being capable of recognizing the T-cell receptor recognized by the parent peptide but with less affinity;

(viii) a nucleotide sequence encoding a pathogenic self-antigen of (vi) or a peptide or a modified peptide of (vii);

(ix) T cells activated by an agent of (i), (vi) or (vii); or

(x) any combination of (i) – (ix).

24. The use according to claim 23, wherein said agent is Copolymer 1, a Copolymer 1-related peptide or a Copolymer 1-related polypeptide.

25. The use according to claim 24, wherein said agent is Copolymer 1.

26. The use according to claim 24, wherein said Copolymer 1-related polypeptide is a random copolymer comprising one amino acid residue selected from each of at least three of the following groups:

(a) lysine and arginine;
(b) glutamic acid and aspartic acid;
(c) alanine and glycine; and
(d) tyrosine and tryptophan.

27. The use according to claim 26, wherein said random copolymer consists of four different amino acid residues, each from a different one of the groups (a) to (d).

28. The use according to claim 27, wherein said four different amino acid residues are alanine, glutamic acid, lysine and tyrosine.

29. The use according to claim 26, wherein said random copolymer consists of three different amino acid residues, each from a different one of three groups (a) to (d).

30 The use according to claim 29, wherein said random copolymer is a terpolymer selected from the group consisting of YAK, YEK, KEA and YEA.

31 The use according to claim 24, wherein said agent is a Copolymer 1-related peptide selected from the peptides represented by SEQ ID Nos. 1-32.

32. The use according to claim 23, wherein said agent is T cells activated by Copolymer 1.

33. The use according to claim 23, wherein said agent is IL-4.

34. The use according to claim 33, wherein IL-4 is for nasal administration.

35. The use according to claim 23, wherein said agent is IL-4 activated dendritic cells, monocytes, bone marrow-derived myeloid cells or peripheral blood mononuclear cells autologous or from a matched donor.

36. The use according to claim 23, wherein said agent is bone marrow-derived myeloid cells or peripheral blood-derived myeloid cells activated with IL-13 or with up to 20 ng/ml IFN-γ.

37. The use according to claim 23, wherein said pathogenic self-antigen associated with a T-cell-mediated specific autoimmune disease of the eye is a pathogenic uveitogenic antigen selected from the group consisting of human interphotoreceptor retinoid-binding protein (IRBP) (SEQ ID NO: 33), bovine IRBP (SEQ ID NO: 34), human S-antigen (S-Ag) (SEQ ID NO: 35), bovine S-Ag (SEQ ID NO: 36) and human rhodopsin (SEQ ID NO: 37).

38. The use according to claim 37, wherein said agent is selected from the group consisting of:

(a) a peptide which sequence is comprised within the sequence of bovine IRBP (SEQ ID NO: 34);

(b) a peptide obtained by modification of the peptide of (a), which modification consists in the replacement of one or more amino acid residues of the peptide by different amino acid residues, said modified peptide still being capable of recognizing the T-cell receptor recognized by the parent peptide but with less affinity (hereinafter "modified peptide");

(c) a nucleotide sequence encoding human IRBP (SEQ ID NO: 33) or bovine IRPB, a peptide of (a), or a modified peptide of (b) ; and

(d) T cells activated by an agent selected from the group consisting of human or bovine IRPB, a peptide of (a), and a modified peptide of (b).

39. The use according to claim 38, wherein said peptide (b) is selected from the group consisting of the peptides:

ADGSSWEGVGVVPDV (SEQ ID NO: 38);

PTARSVGAADGSSWEGVGVVPDV (SEQ ID NO: 39); and
HVDDTDLYLTIPTARSVGAADGS (SEQ ID NO: 40).

40. The use according to claim 37, wherein said agent is selected from the group consisting of:

(a) a peptide which sequence is comprised within the sequence of bovine S-Ag (SEQ ID NO: 36);

(b) a peptide obtained by modification of the peptide of (a), which modification consists in the replacement of one or more amino acid residues of the peptide by different amino acid residues, said modified peptide still being capable of recognizing the T-cell receptor recognized by the parent peptide but with less affinity (hereinafter "modified peptide");

(c) a nucleotide sequence encoding human S-Ag (SEQ ID NO: 35) or bovine S-Ag, a peptide of (a), or a modified peptide of (b) ; and

(d) T cells activated by an agent selected from the group consisting of human or bovine S-Ag, a peptide of (a), and a modified peptide of (b).

41. The use according to claim 40, wherein said peptide (b) is selected from the group consisting of the peptides:

TSSEVATE (SEQ ID NO:41);
DTNLASST (SEQ ID NO:42);
DTNLASSTIIKEGIDKTV (SEQ ID NO:43);
VPLLANNRERRGIALDGKIKHE (SEQ ID NO:44);
TSSEVATEVPFRLMHPQPED (SEQ ID NO:45);
SLTKTLTLVPLLANNRERRG (SEQ ID NO:46);
SLTRTLTLLPLLANNRERAG (SEQ ID NO:47);
KEGIDKTVMGILVSYQIKVKL (SEQ ID NO:48); and
KEGIDRTVLGILVSYQIKVKL (SEQ ID NO:49).

42. The use according to claim 40, wherein said modified peptide (c) is selected from the group consisting of the peptides:

TSSEAATE	(SEQ ID NO:50); and
DTALASST	(SEQ ID NO:51).

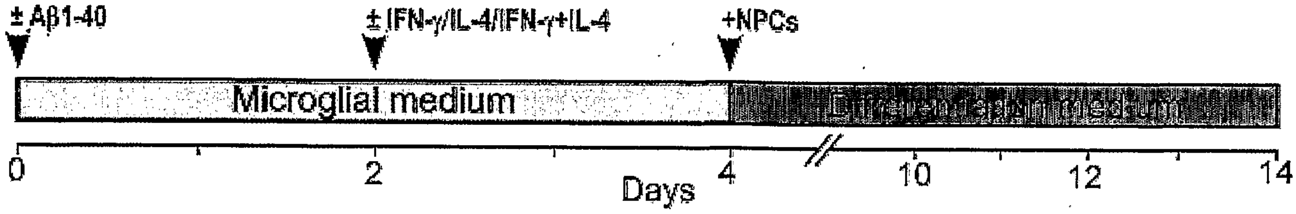

± Aβ1-40
± IFN-γ/IL-4/IFN-γ+IL-4
+NPCs
Microglial medium
0
2
4
10
12
14
Days

Fig. 1A

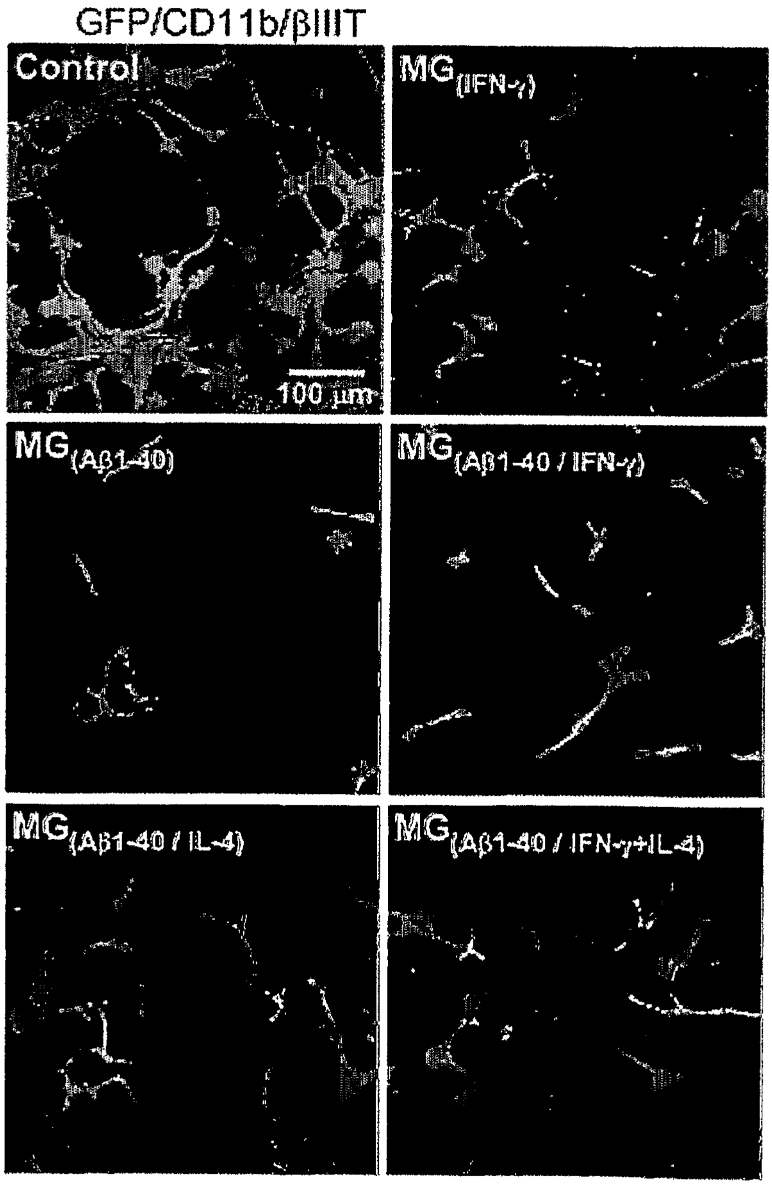

GFP/CD11b/βIIIT
Control
100 µm
MG(IFN-γ)
MG(Aβ1-40)
MG(Aβ1-40 / IFN-γ)
MG(Aβ1-40 / IL-4)
MG(Aβ1-40 / IFN-γ+IL-4)

Fig. 1B

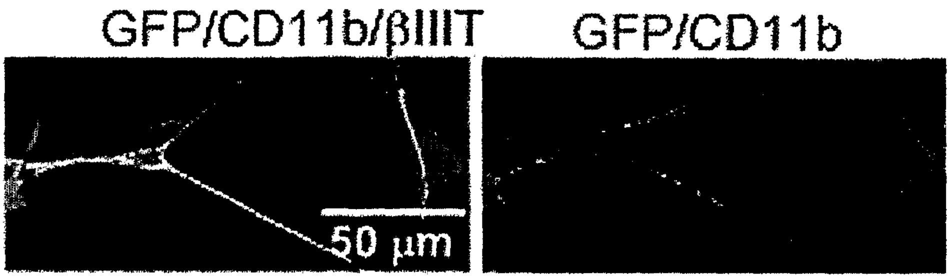

GFP/CD11b/βIIIT
GFP/CD11b
50 μm

Fig. 1C

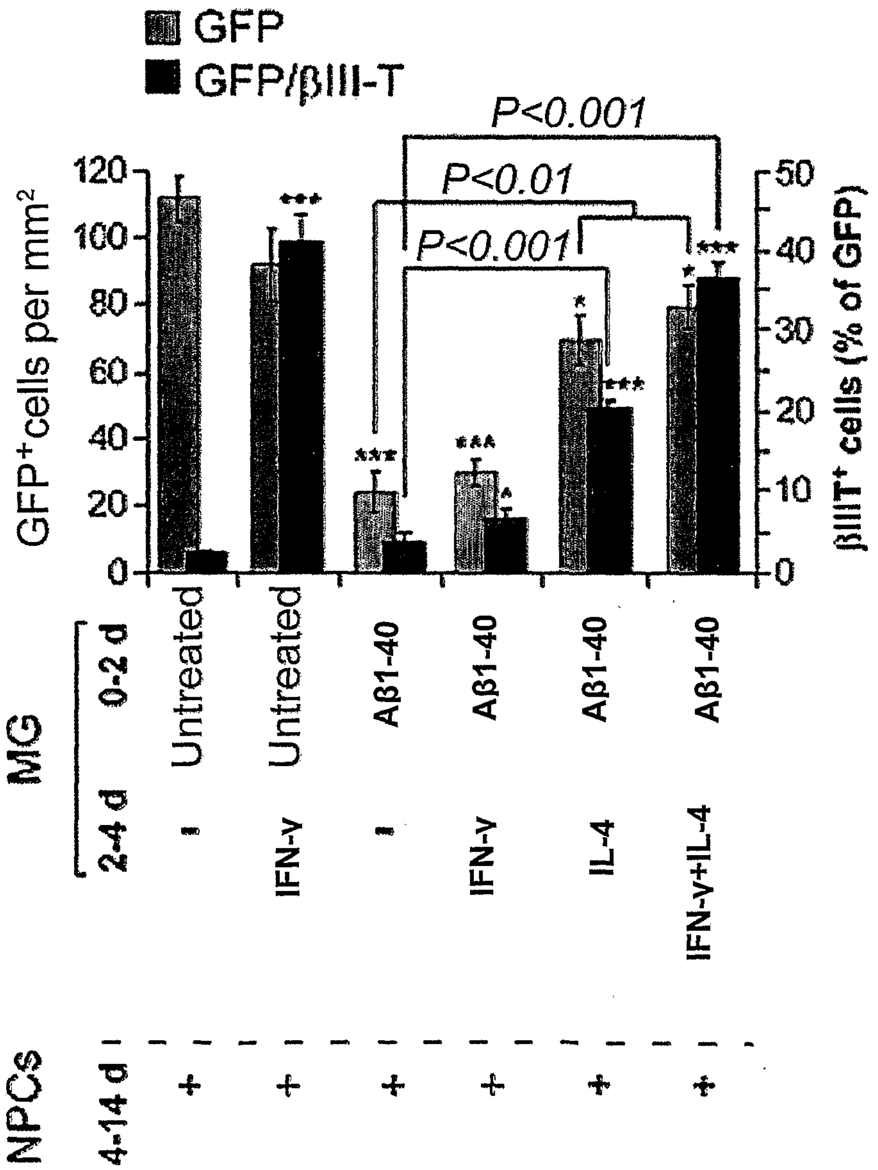

GFP
GFP/βIII-T
P<0.001
P<0.01
P<0.001
GFP⁺cells per mm²
βIIIT⁺ cells (% of GFP)
MG
0-2 d
2-4 d
Untreated
Untreated
Aβ1-40
Aβ1-40
Aβ1-40
Aβ1-40
-
IFN-γ
-
IFN-γ
IL-4
IFN-γ+IL-4
NPCs
4-14 d
+
+
+
+
+
+

Fig. 1D

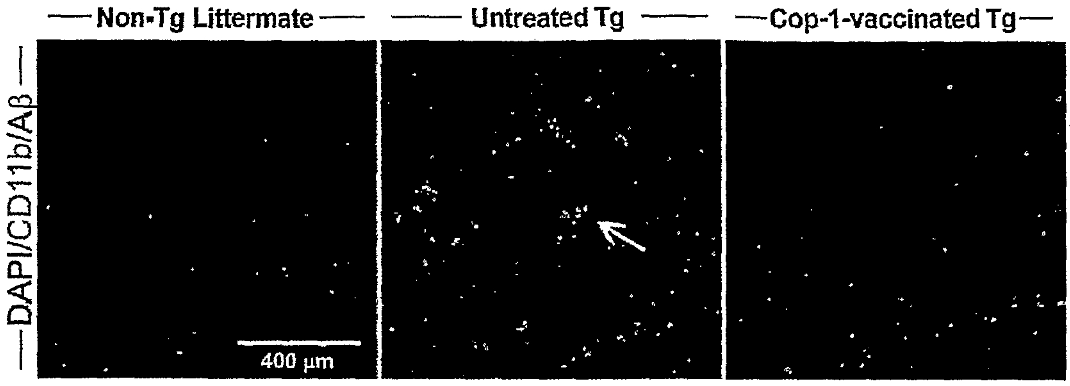


Fig. 2A

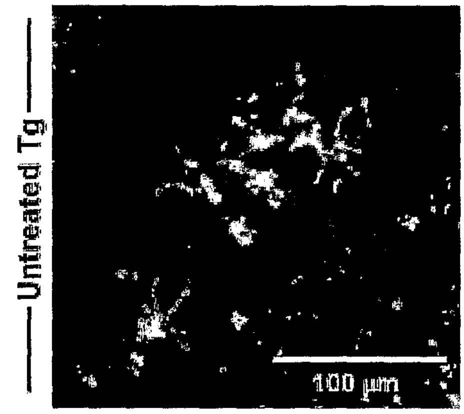


Fig. 2B

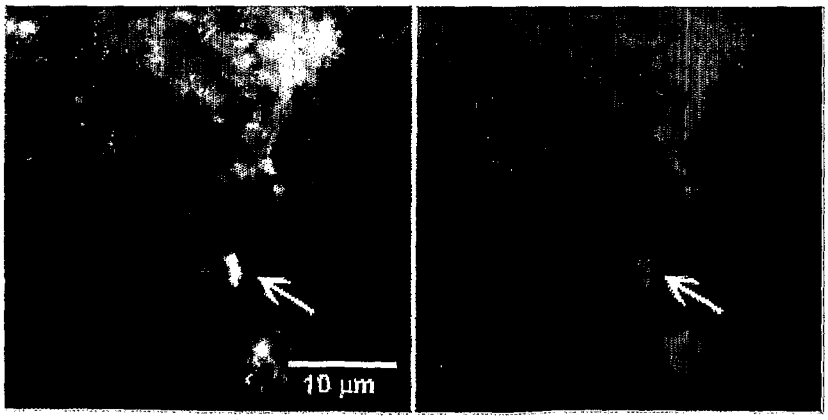


Fig. 2C

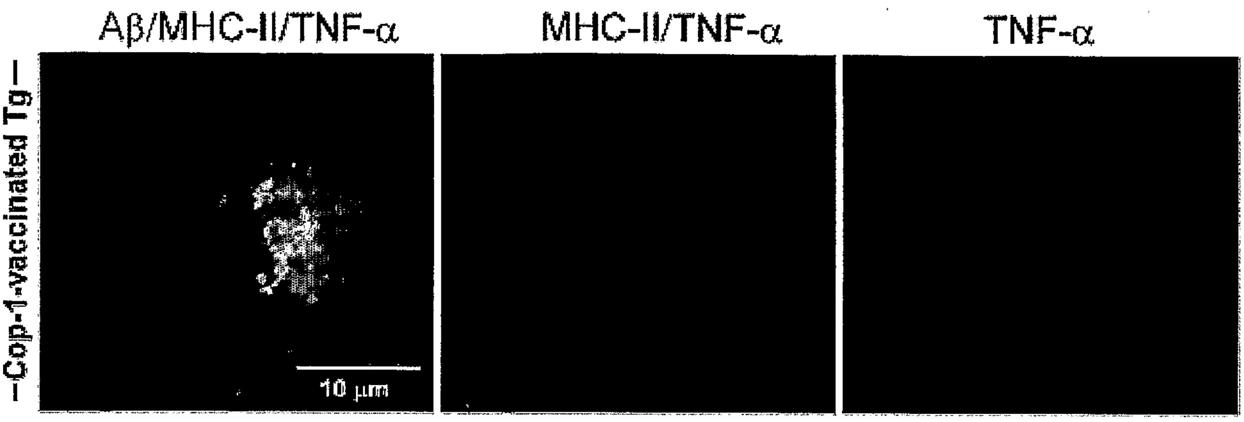

Aβ/MHC-II/TNF-α
MHC-II/TNF-α
TNF-α
Cop-1-vaccinated Tg
10 μm

Fig. 2D

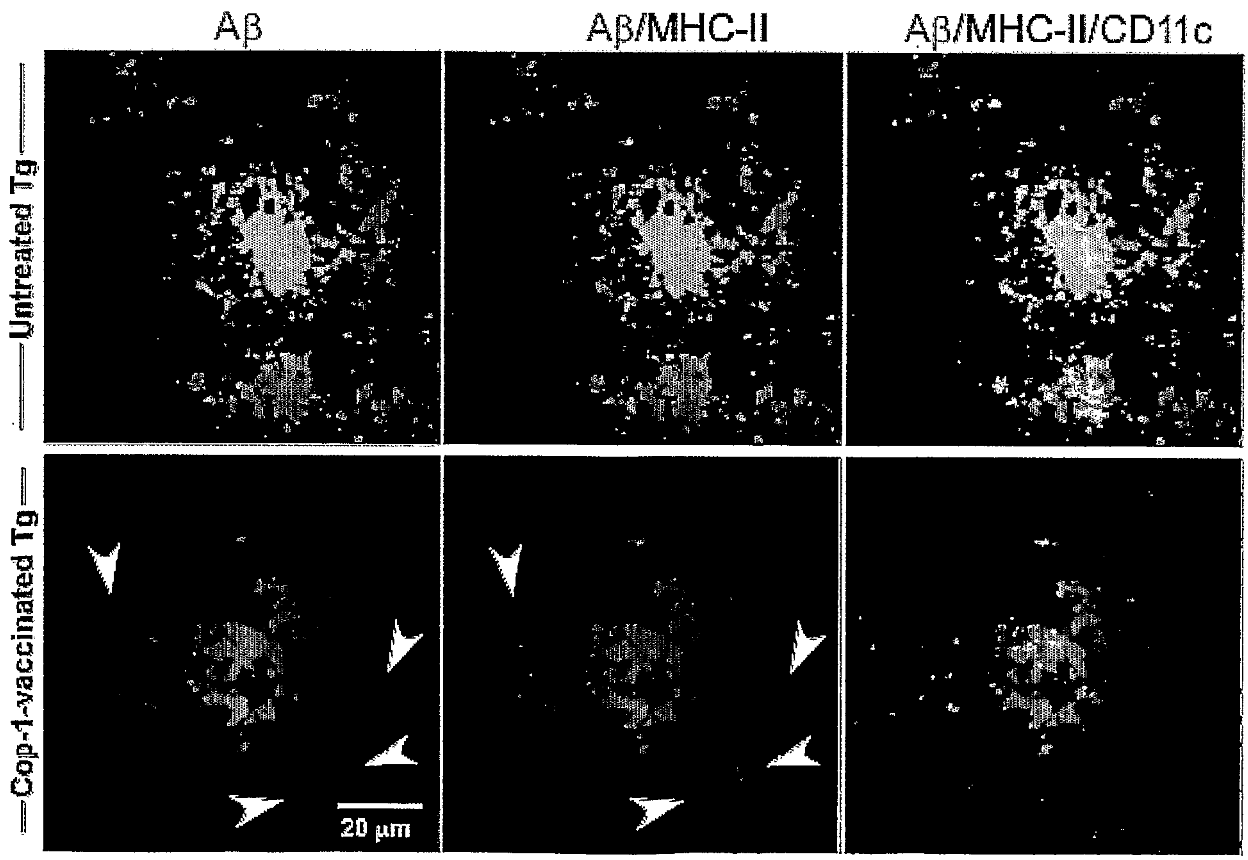

Aβ
Aβ/MHC-II
Aβ/MHC-II/CD11c
Untreated Tg
Cop-1-vaccinated Tg
20 μm

Fig. 2E

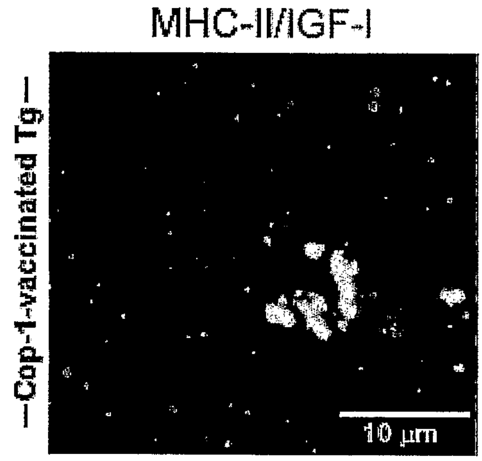


Fig. 2F

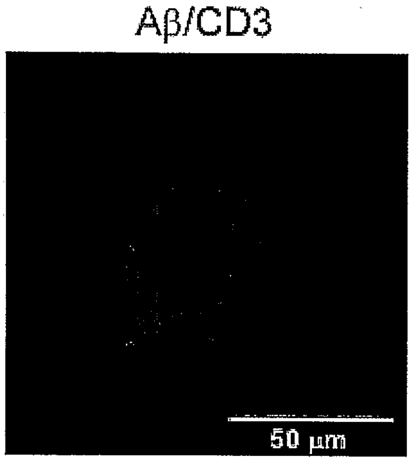


Fig. 2G

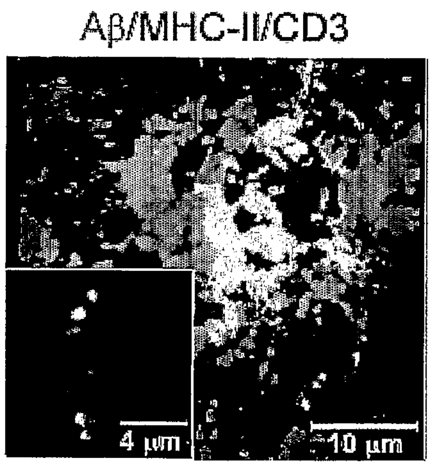


Fig. 2H

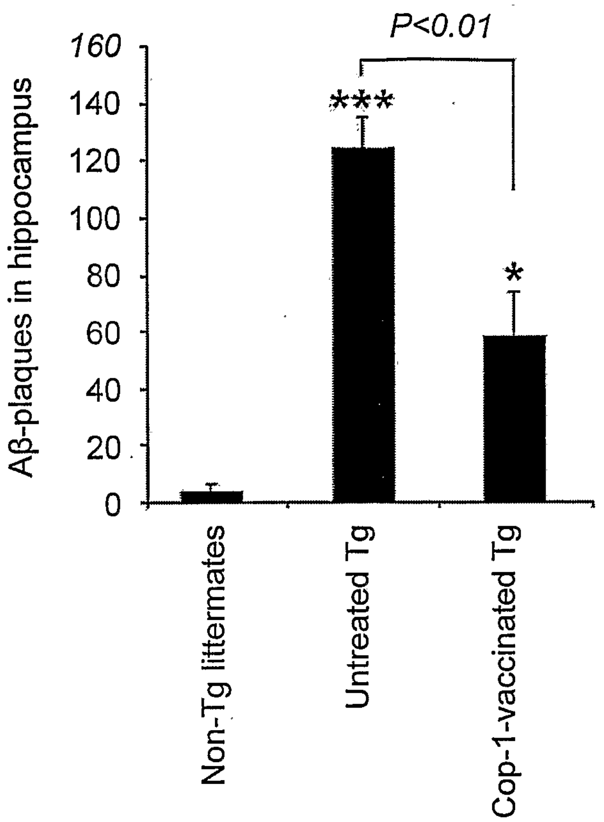


Fig. 2I

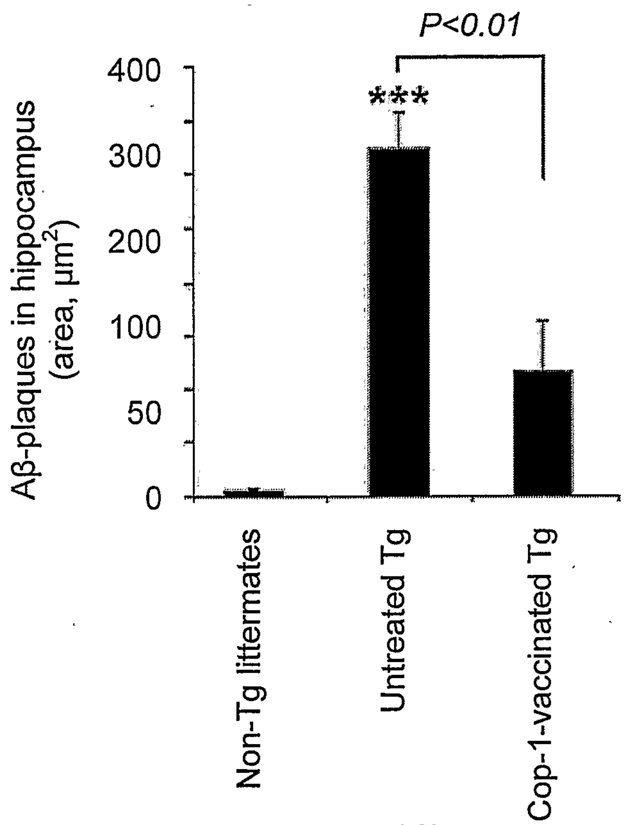


Fig. 2J

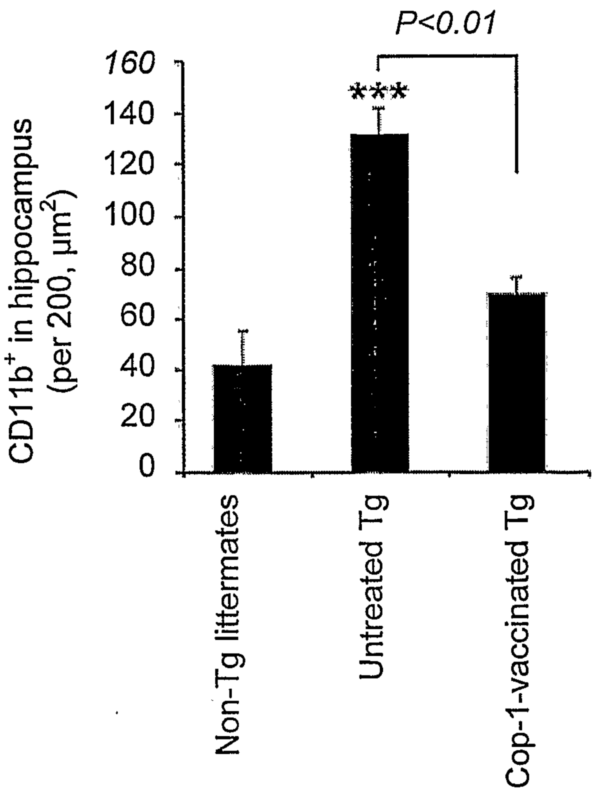


Fig. 2K

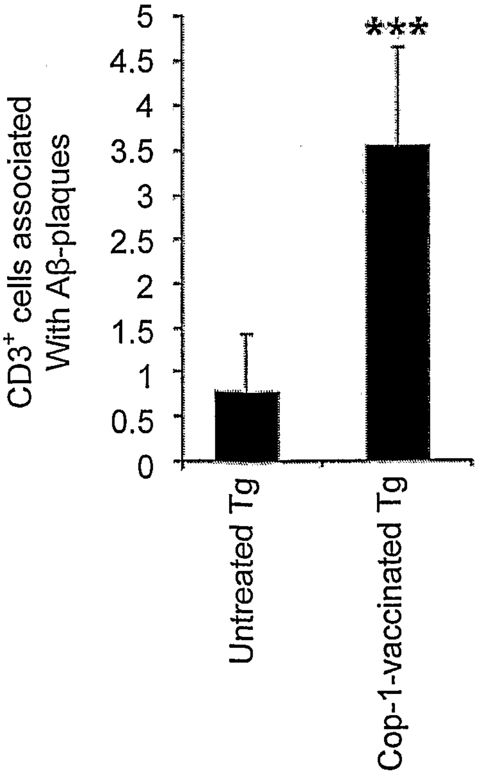


Fig. 2L

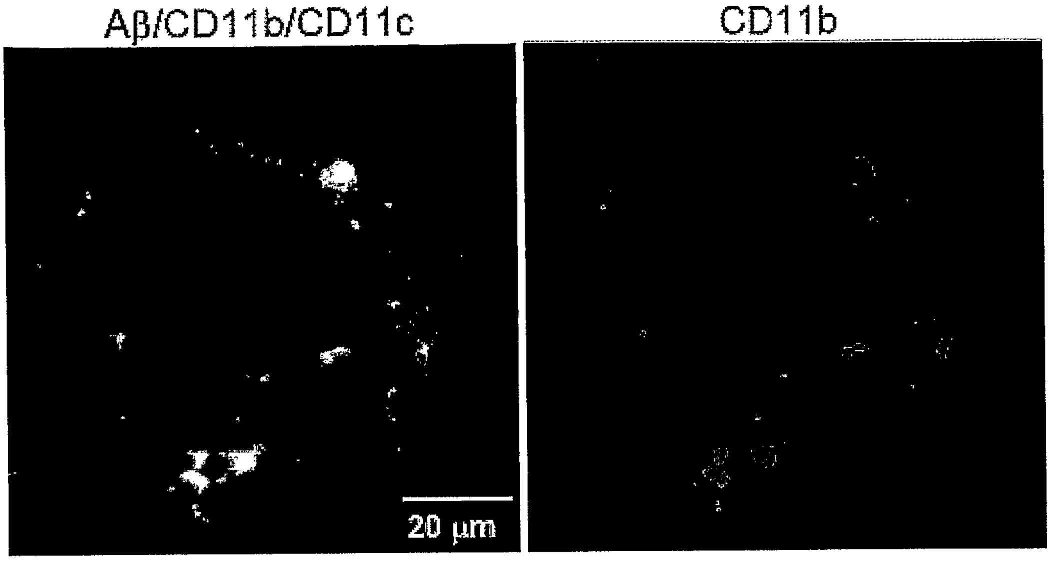


Fig. 3A

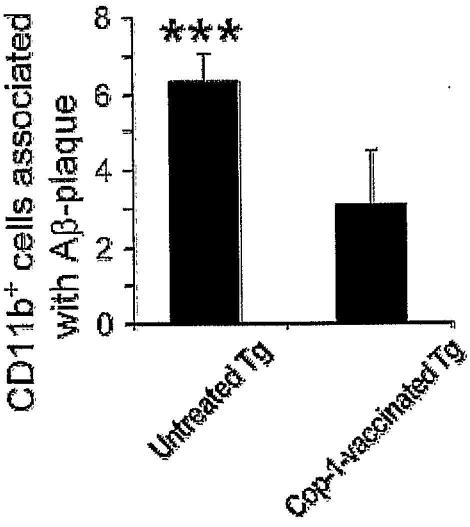


Fig. 3B

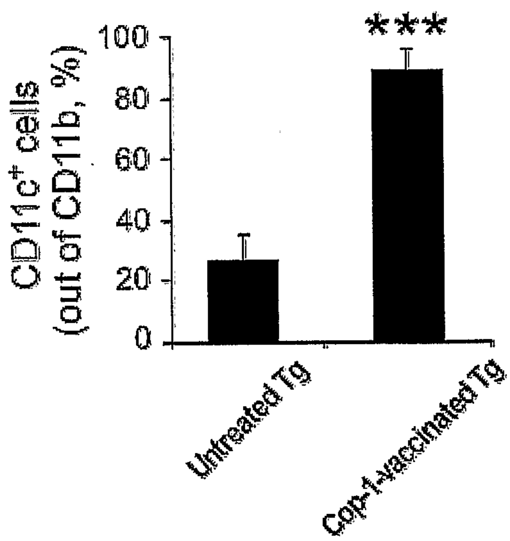


Fig. 3C

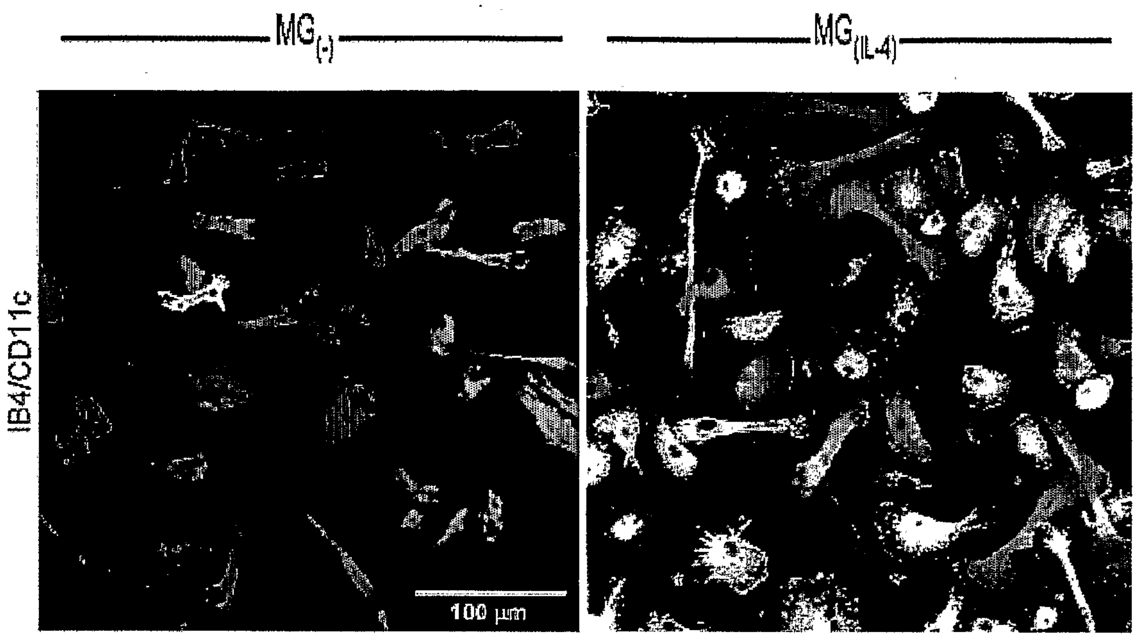

MG(-)
MG(IL-4)
IB4/CD11c
100 µm

Fig. 4A

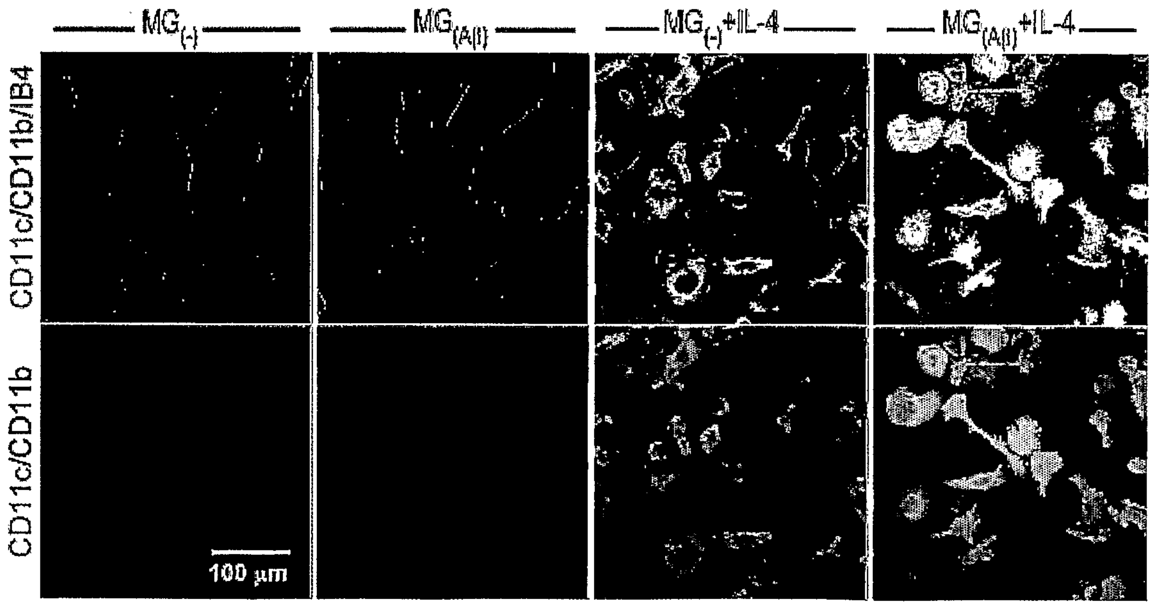

MG(-)
MG(Aβ)
MG(-)+IL-4
MG(Aβ)+IL-4
CD11c/CD11b/IB4
CD11c/CD11b
100 µm

Fig. 4B

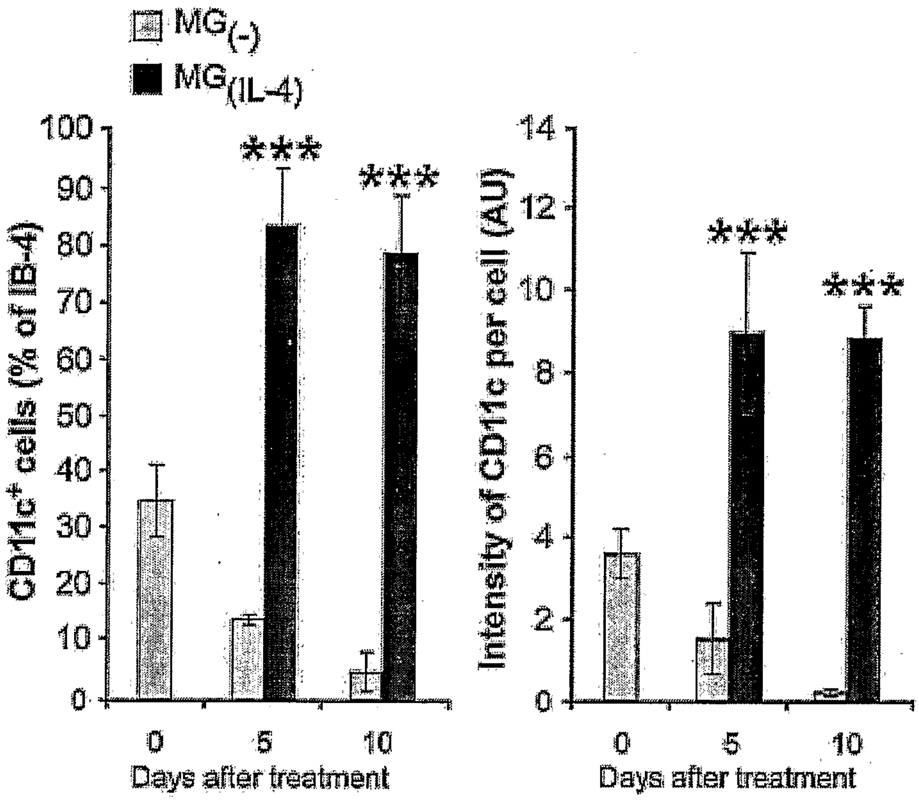

MG(-)
MG(IL-4)
CD11c+ cells (% of IB-4)
Intensity of CD11c per cell (AU)
***
Days after treatment
0
5
10

Fig. 4C

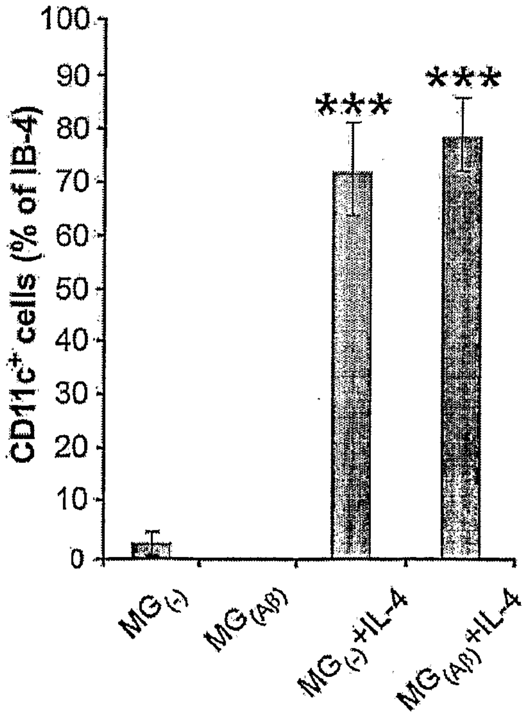

CD11c+ cells (% of IB-4)
***
MG(-)
MG(Aβ)
MG(-)+IL-4
MG(Aβ)+IL-4

Fig. 4D

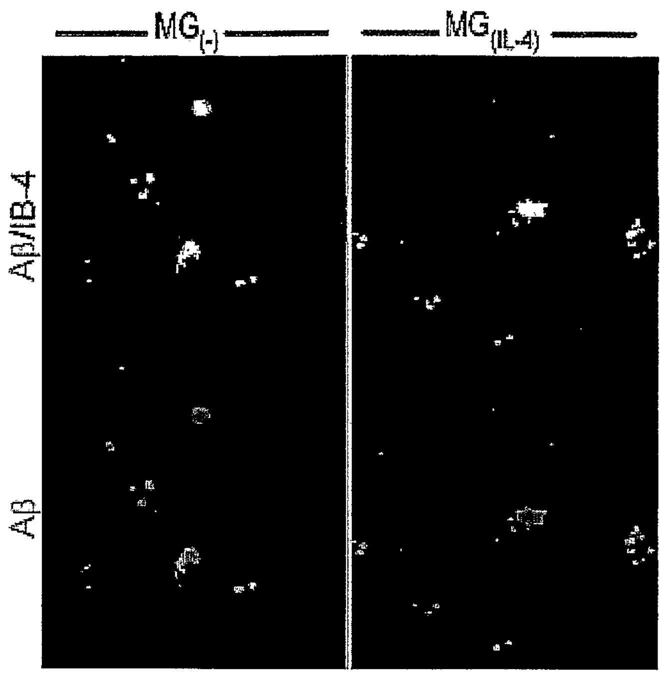

MG(-)
MG(IL-4)
Aβ/IB-4
Aβ

Fig. 5A

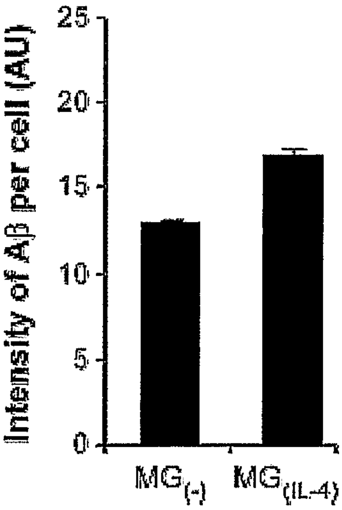

Intensity of Aβ per cell (AU)
25
20
15
10
5
0
MG(-)
MG(IL-4)

Fig. 5B

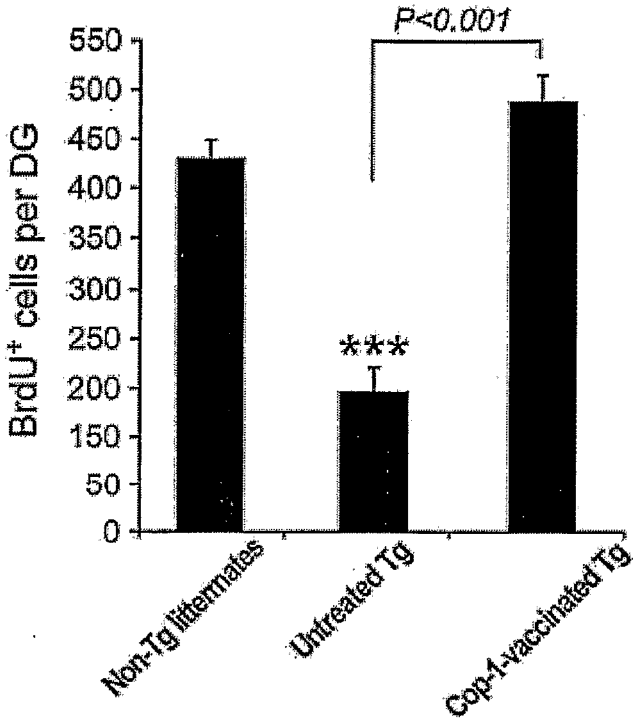

P<0.001
550
500
450
400
350
300
250
200
150
50
0
BrdU⁺ cells per DG
***
Non-Tg littermates
Untreated Tg
Cop-1-vaccinated Tg

Fig. 6A

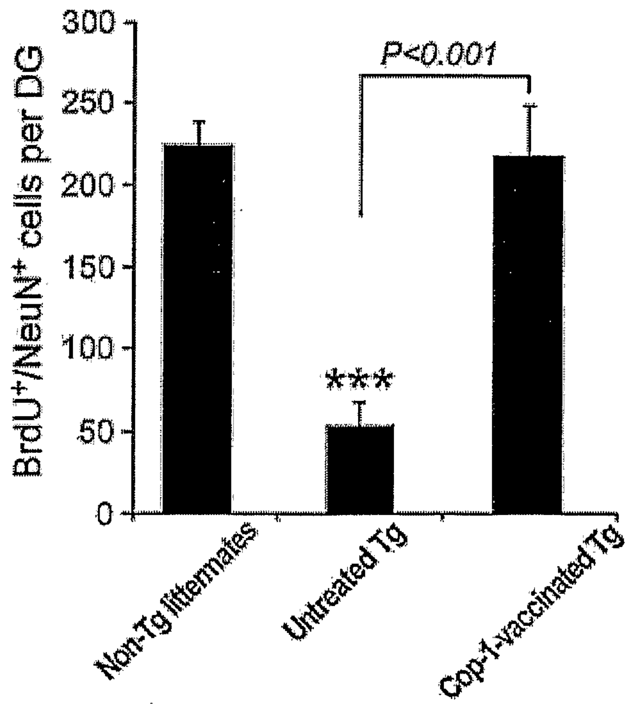

300
250
200
150
100
50
0
BrdU⁺/NeuN⁺ cells per DG
P<0.001
***
Non-Tg littermates
Untreated Tg
Cop-1-vaccinated Tg

Fig. 6B

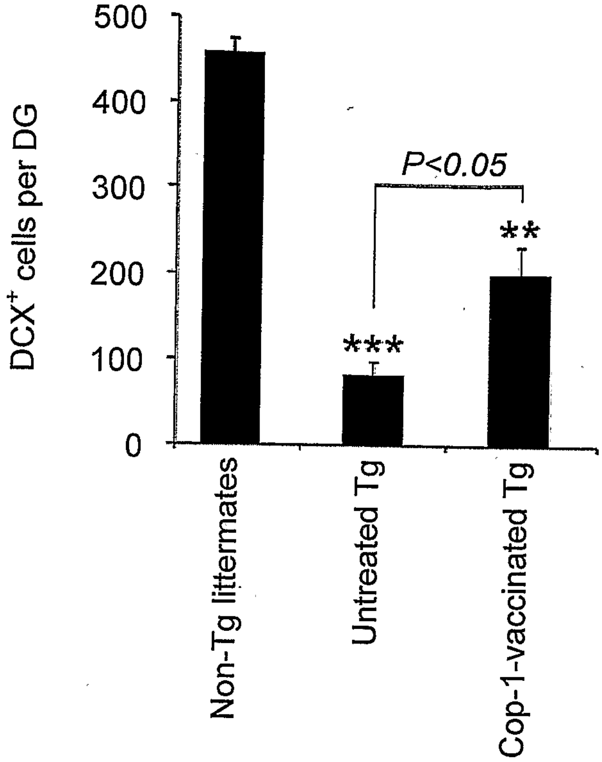


Fig. 6C

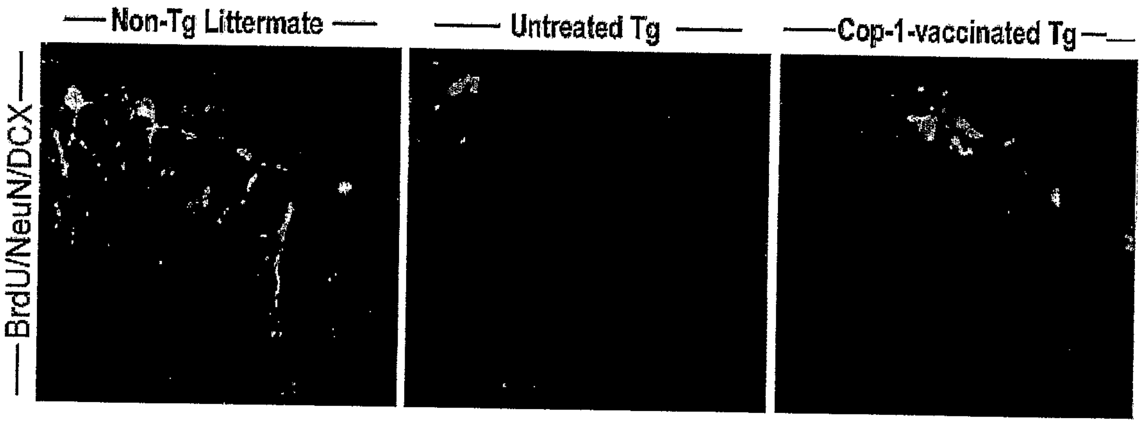


Fig. 6D

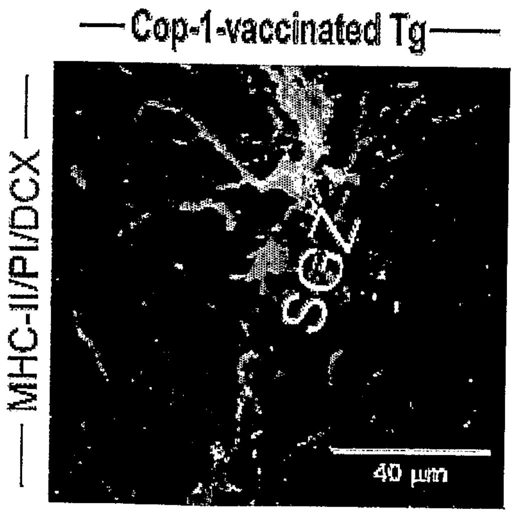
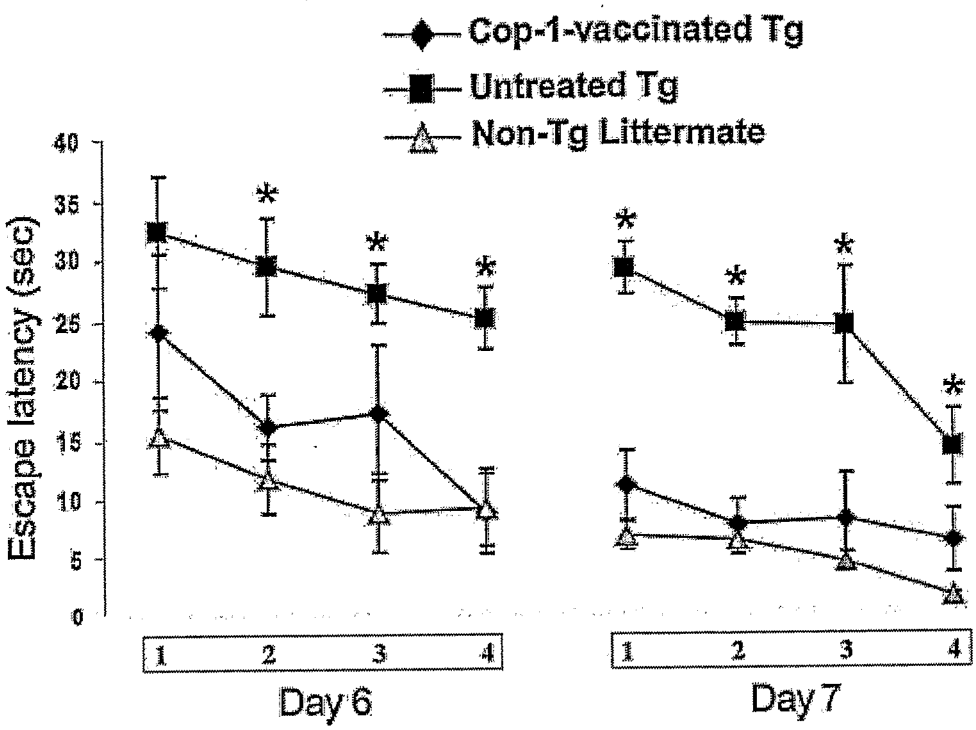
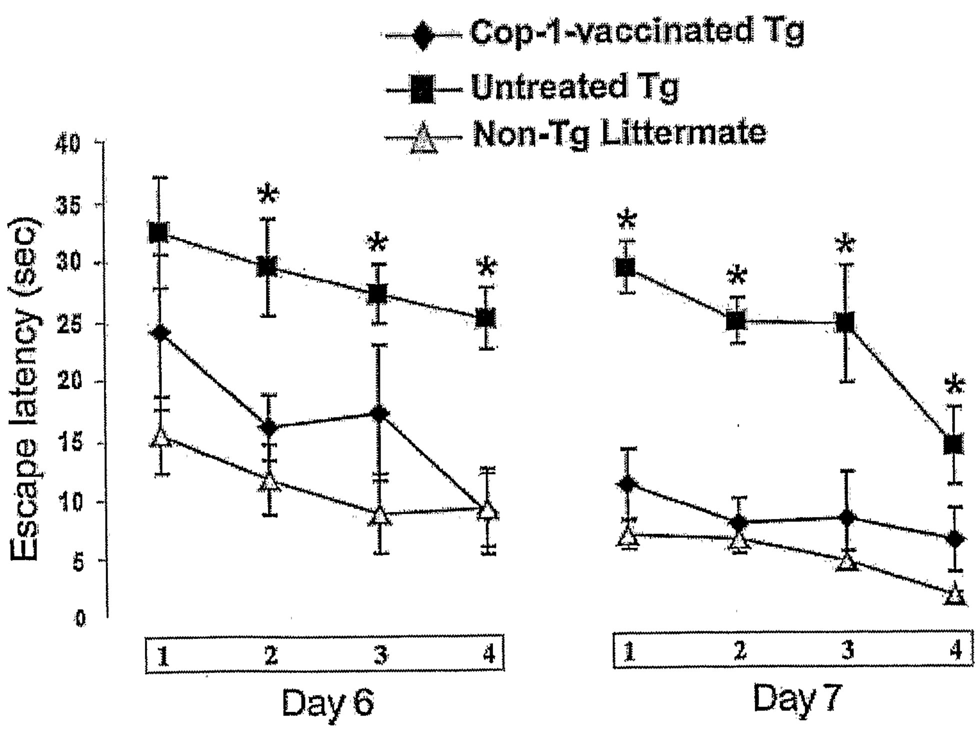


Fig. 6E



Cop-1-vaccinated Tg
Untreated Tg
Non-Tg Littermate
Escape latency (sec)
40
35
30
25
20
15
10
5
0
*
1
2
3
4
Day 6
Day 7

Fig. 7A



Cop-1-vaccinated Tg
Untreated Tg
Non-Tg Littermate
Escape latency (sec)
40
35
30
25
20
15
10
5
0
*
1
2
3
4
Day 6
Day 7

Fig. 7B

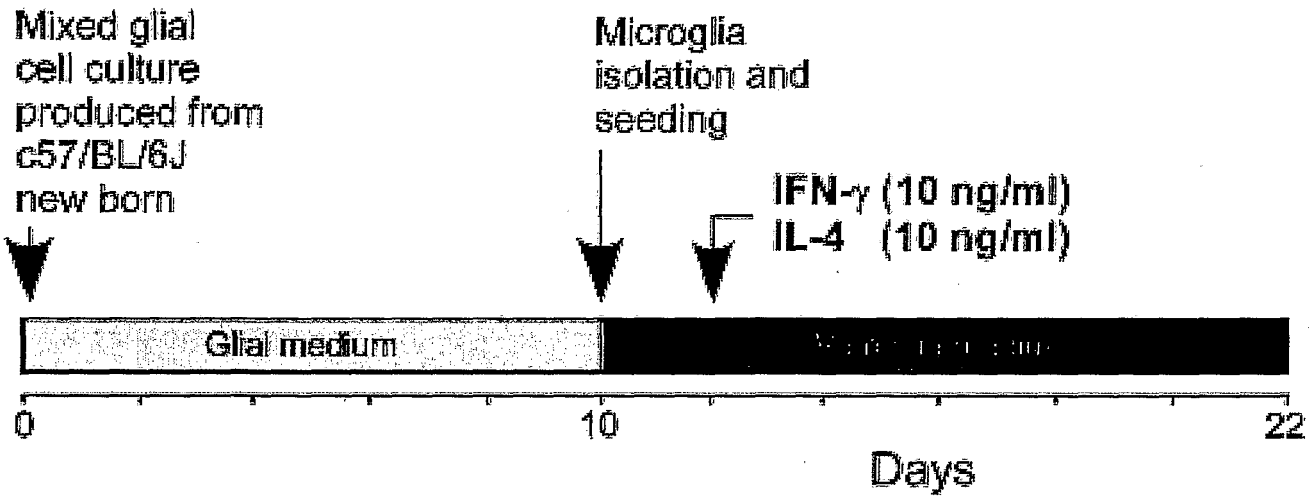


Fig. 8A

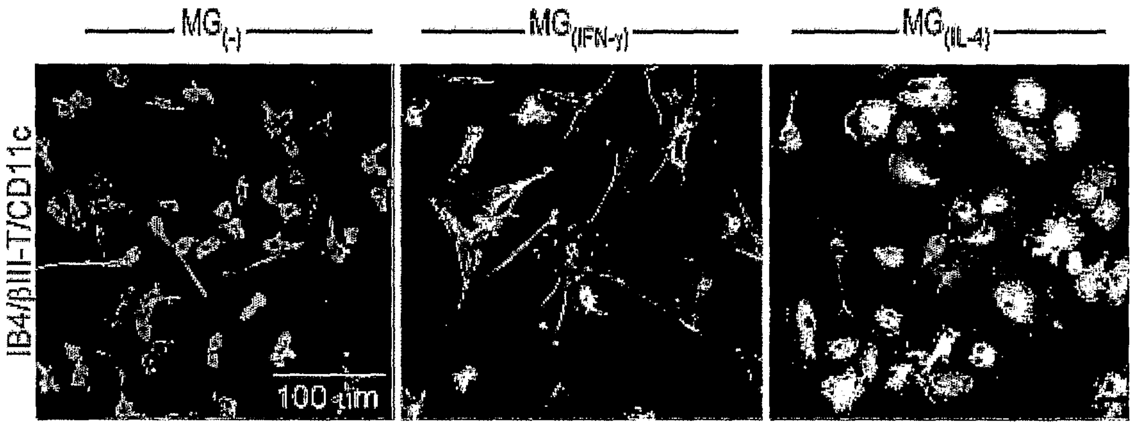


Fig. 8B

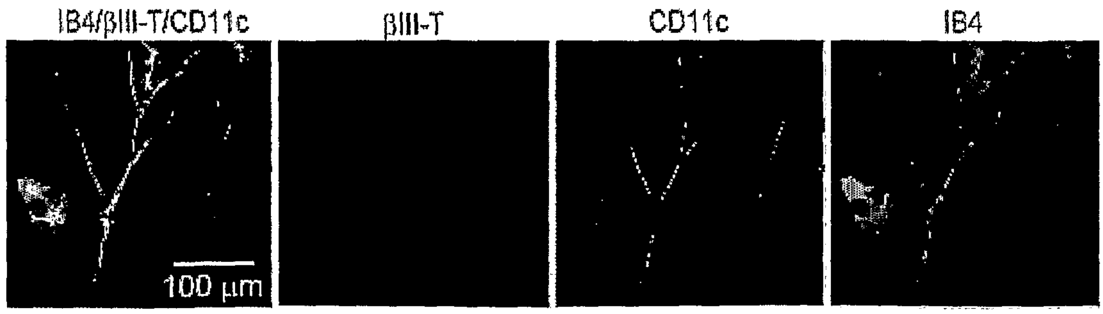


Fig. 8C

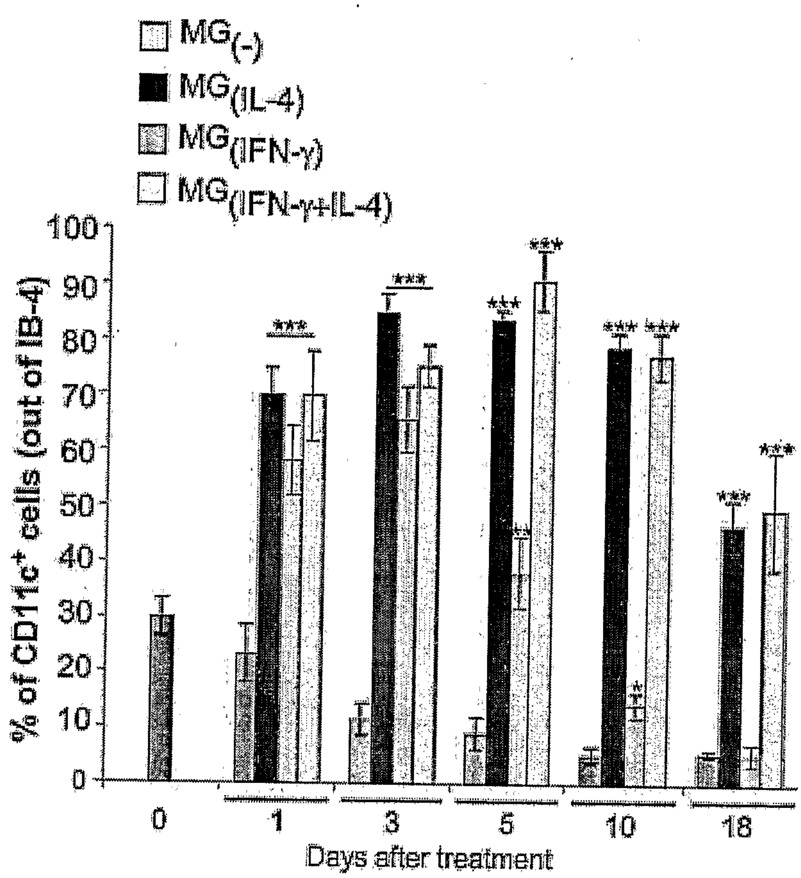

MG(-)
MG(IL-4)
MG(IFN-γ)
MG(IFN-γ+IL-4)
% of CD11c+ cells (out of IB-4)
100
90
80
70
60
50
40
30
20
10
0
***
0
1
3
5
10
18
Days after treatment

Fig. 8D