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(54) **Title**: ANTAGONISTIC ANTIBODIES AGAINST OSCAR

(57) **Abstract**: The present disclosure relates to antagonistic antibodies against OSCAR. The present disclosure furthermore relates to use of such antibodies.

ANTAGONISTIC ANTIBODIES AGAINST OSCAR

TECHNICAL FIELD

The present invention relates to compounds useful in treatment and prophylaxis of diseases such as autoimmune inflammatory diseases and bone resorption related disorders.
5 The present invention furthermore relates to OSCAR antagonists as well as use of such compounds.

BACKGROUND

Osteoclast-associated receptor (OSCAR) is an activating receptor expressed on
10 osteoclasts and myeloid-derived cells, including monocytes, macrophages, neutrophils and dendritic cells. It was reported that OSCAR is up-regulated in the synovial tissue as well as in circulating $CD14^{+}$ cells from rheumatoid arthritis patients. Triggering of OSCAR signalling leads to secretion of pro-inflammatory cytokines and chemokines from monocytes and dendritic cells, promotes differentiation of osteoclasts and hence may be causally involved in
15 the pathogenesis of the inflammation-induced bone resorption. Collagens type I, II, III, and IV have been shown to act as ligands for OSCAR (WO2010040998).

No OSCAR antagonists suitable for therapeutic use in patients with diseases associated with bone resorption, e.g. rheumatoid arthritis or other inflammatory diseases have been disclosed thus far.

20 **SUMMARY**

The present invention thus relates to OSCAR antagonists, preferably OSCAR antibodies, as well as use of such compounds in treatment of various diseases such as e.g. autoimmune inflammatory diseases. Such antagonists have the ability to reduce inflammation and/or the ability to modulate osteoclast-mediated bone resorption.
25 The present invention furthermore relates to methods for inducing collagen dependent maturation of monocyte-derived Dendritic Cells.

DESCRIPTION

The present invention relates to OSCAR antagonists that are useful in connection with treatment of patients suffering from autoimmune inflammatory diseases such as e.g.
30 rheumatoid arthritis. The compounds of the invention may also be used in treatment of other disorders associated with bone resorption.

Brief description of drawings:

Figure 1: Anti-OSCAR mAb 13F5A5 inhibit osteoclast formation from normal monocytes

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Definitions:

OSCAR: the osteoclast-associated receptor (OSCAR) is a member of the leukocyte receptor complex (LRC) protein family that plays critical roles in the regulation of both innate
10 and adaptive immune responses. OSCAR is expressed in pre-osteoclasts and mature osteoclasts (OC) and promotes OC differentiation from the bone marrow derived precursors. In contrast to mouse OSCAR which expression is restricted to OC, the human receptor is also found in monocytes, macrophages, dendritic cells and neutrophils. In the latter cell types triggering of OSCAR can lead to secretion of pro-inflammatory mediators and promote
15 differentiation of OC from monocyte-derived precursors. The best studied isoform of the OSCAR protein (further referred as “OSCAR”, encoded by mRNA with Genebank accession number AF391162), is a single-span trans-membrane protein comprising 263 amino acids residues (SEQ ID NO 1). It associates with FcRγ which serves as a signalling subunit of the receptor complex. Sequences of six additional putative OSCAR isoforms can be found in
20 various databases (Uniprot accession number Q81YS5-1, Q81YS5-2, Q81YS5-3, Q81YS5-4, Q81YS5-5, Q81YS5-6, Q81YS5-7). All isoforms seem to be resulting from the alternative splicing of a common RNA precursor. Some isoforms are predicted to lack a transmembrane domain due to a frame shift mutation and thus encode for putative secreted proteins. Recombinant soluble proteins comprising e.g. the extracellular domain of OSCAR (OSCAR-
25 ECD) and naturally occurring isoforms OSCAR-S1a and OSCAR-S1b can be generated. The amino acid sequence of human OSCAR-ECD is shown in SEQ ID NO 2. The amino acid sequence of human OSCAR-S1a is shown in SEQ ID NO 3. The amino acid sequence of human OSCAR-S1b is shown in SEQ ID NO 4. “OSCAR variants” refer to proteins having OSCAR activity but comprise one or more alterations in comparison with wt OSCAR, such as
30 e.g. one or more amino acid substitutions/deletions/additions.

SEQ ID NO 1: Human OSCAR (putative signal peptide marked with bold)

MALVLILQLLTLWPLCHTDITPSVPPASYHPKPWLGAQPATVVTPGVNVTLRCRA PQPAWRFGLFKPGEIAPLLFRDVSSELAEFFLEEVTPAQGGSYRCCYRRPDWGPGVWSQP SDVLELLVTEELPRPSLVALPGPVVGPGANVSLRCAGRLRNMSFVLYREGVAAPLQYRHSA

QPWADFTLLGARAPGTYSCYYHTPSAPYVLSQRSEVLVISWEDSGSSDYTRGNLVRLGLAG
LVLISLGALVTFDWRSQNRAPAGIRP

SEQ ID NO 2: Human OSCAR-ECD

5 **MALVLILQLLTLWPLCHT**DITPSVPPASYHPKPWLGAQPATVVTPGVNVTLRCRAP
QPAWRFGLFKPGEIAPLLFRDVSSELAEFFLEEVTPAQGGSYRCCYRRPDWGPGVWSQPS
DVLELLVTEELPRPSLVALPGPVVGPGANVSLRCAGRLRNMSFVLYREGVAAPLQYRHSAQ
PWADFTLLGARAPGTYSCYYHTPSAPYVLSQRSEVLVISWEDSGSSDYTR

10 **SEQ ID NO 3: Human OSCAR-S1a**

MALVLILQLLTLWPLCHTDITPSVPPASYHPKPWLGAQPATVVTPGVNVTLRCRAP
QPAWRFGLFKPGEIAPLLFRDVSSELAEFFLEEVTPAQGGSYRCCYRRPDWGPGVWSQPS
DVLELLVTEELPRPSLVALPGPVVGPGANVSLRCAGRLRNMSFVLYREGVAAPLQYRHSAQ
PWADFTLLGARAPGTYSCYYHTPSAPYVLSQRSEVLVISWEGEGPEARPASSAPGMQAPG
15 PPPSDPGAQAPSLSSFRPRGLVLQPLLPQTQDSWDPAPPPSDPGV

SEQ ID NO 4: Human OSCAR-S1b

MALVLILQLLTLWPLCHTDITPSVAIIVPPASYHPKPWLGAQPATVVTPGVNVTLRC
RAPQPAWRFGLFKPGEIAPLLFRDVSSELAEFFLEEVTPAQGGSYRCCYRRPDWGPGVWS
20 QPSDVLELLVTEELPRPSLVALPGPVVGPGANVSLRCAGRLRNMSFVLYREGVAAPLQYRH
SAQPWADFTLLGARAPGTYSCYYHTPSAPYVLSQRSEVLVISWEGEGPEARPASSAPGMQ
APGPPPSDPGAQAPSLSSFRPRGLVLQPLLPQTQDSWDPAPPPSDPGV

WO2010040998 discloses that collagen proteins/peptides can act as ligands for
25 OSCAR. WO2010040998 furthermore discloses that OSCAR binding to collagen peptides
stimulates the activation and/or differentiation of OC. The effect can be blocked by the
11.1CN5 antibody. The 11.1CN5 antibody is an anti-human OSCAR mAb and it is an R-
phycoerythrin conjugated antibody provided in a composition comprising sodium azide
(https://www.beckmancoulter.com/wsrportal/search/Anti-OSCAR-
30 PE/#2/10//0/25/1/0/asc/2/Anti-OSCAR-PE//0/1//0/).

Binding of antibodies and ligands to OSCAR can be measured by ELISA and SPR
using recombinant proteins or by FACS and FMAT using transfected cells or cells naturally
expressing OSCAR, such as e.g. monocytes. OSCAR activation upon ligand binding can be
assessed by e.g. analysing expression of cell surface markers using FACS, by detecting

cytokine release using ELISA or Bio-Plex; by detecting a reporter gene activity or by using an osteoclast formation assay.

Collagen: Collagen is the main component of connective tissue and extracellular matrix and are the most abundant protein in mammals. Collagen type I is mainly found in skin, tendon, vascular, ligature and bone (major protein component of bone). Collagen type II is mainly found in cartilage (major protein component of cartilage). Collagen type III is mainly found in reticulate (major component of reticular fibres). Collagen types I, II, III, and IV are the "fibrillar collagens". Collagen I may herein be referred to as "ColI", collagen II as "ColII, collagen III as "ColIII" and collagen IV as "ColIV".

Therapeutic Applications: The compounds according to the invention can be used in the treatment of diseases involving an inappropriate or undesired immune response (immunological disorders), such as inflammation, autoimmunity, and conditions involving such mechanisms as well as graft vs. host disease. In one embodiment, such disease or disorder is an autoimmune and/or inflammatory disease. Examples of such autoimmune and/or inflammatory diseases are systemic lupus erythematosus (SLE), rheumatoid arthritis (RA), inflammatory bowel disease (IBD) (including ulcerative colitis (UC) and Crohn's disease (CD)), multiple sclerosis (MS), scleroderma, type 1 diabetes (T1 D), pemphigus vulgaris (PV), psoriasis, atopic dermatitis, celiac disease, Hashimoto's thyroiditis, Graves' disease, Sjogren's syndrome, Guillain-Barre syndrome, Goodpasture's syndrome, Addison's disease, Wegener's granulomatosis, primary biliary sclerosis, sclerosing cholangitis, autoimmune hepatitis, polymyalgia rheumatica, Raynaud's syndrome, temporal arteritis, giant cell arteritis, autoimmune hemolytic anemia, pernicious anemia, polyarteritis nodosa, Behcet's disease, primary bilary cirrhosis, uveitis, myocarditis, rheumatic fever, ankylosing spondylitis, glomerulonephritis, sarcoidosis, dermatomyositis, myasthenia gravis, polymyositis, alopecia areata, and vitilgo. The compounds according to the invention can furthermore be used in treatment of bone resorption related disorders such as e.g. osteoarthritis, osteoporosis, psoriatic arthritis, etc.

Rheumatoid arthritis (RA): RA is a systemic autoimmune disease that affects the entire body and is one of the most common forms of arthritis. It is characterized by the inflammation of the synovial membrane lining a joint, which causes pain, stiffness, warmth, redness and swelling. Inflammatory cells release enzymes that may digest bone and cartilage. As a result of RA, the inflamed joint lining, the synovium, can invade and damage bone and cartilage leading to joint deterioration and severe pain amongst other physiologic

effects. The involved joint can lose its shape and alignment, resulting in pain and loss of movement. Anti-inflammatory biological therapeutics are now available for the treatment of rheumatoid arthritis (e.g. anti-CD20, a TNF-R antagonist or anti-TNF-α). However, none of these medicaments are specifically reducing bone erosion in RA patients.

5 There are several animal models for RA known in the art. For example, in the collagen-induced arthritis (CIA) model, mice develop chronic inflammatory arthritis that closely resembles human RA.

The term **"treatment"**, as used herein, refers to the medical therapy of any human subject or other animal subject in need thereof. Said subject is expected to have undergone 10 physical examination by a medical or veterinary medical practitioner, who has given a tentative or definitive diagnosis which would indicate that the use of said specific treatment is beneficial to the health of said human or other animal subject. The timing and purpose of said treatment may vary from one individual to another, according to the status of the subject's health. Thus, said treatment may be prophylactic, palliative, symptomatic and/or 15 curative. In terms of the present invention, prophylactic, palliative, symptomatic and/or curative treatments may represent separate aspects of the invention.

The term "**antibody**" herein refers to a protein, derived from a germline immunoglobulin sequence capable of specifically binding to an antigen or a portion thereof. The term includes full length antibodies of any isotype (i.e. IgA, IgD, IgE, IgG, IgM and/or 20 IgY), any antigen binding fragment thereof, and any chain thereof. Examples of various types of molecules being encompassed by the definition of "antibodies" are listed below. Antibodies may be referred to in various ways herein, e.g. "antibody", "Ab", "mAb", "monoclonal antibody", etc, and such terms may be used interchangeably.

Full-length antibodies usually comprise at least four polypeptide chains: that is, two 25 heavy (H) chains and two light (L) chains that are interconnected by disulfide bonds. One immunoglobulin sub-class of particular pharmaceutical interest is the IgG family, which may be sub-divided into isotypes IgG1, IgG2, IgG3 and IgG4. IgG molecules are composed of two heavy chains, usually interlinked by two or more disulfide bonds, and two light chains, ordinarily attached to a heavy chain by a disulfide bond. A heavy chain may comprise a 30 heavy chain variable region (VH) and up to three heavy chain constant (CH) regions: CH1, CH2 and CH3. A light chain may comprise a light chain variable region (VL) and a light chain constant region (CL). VH and VL regions can be further subdivided into regions of hypervariability, termed complementarity determining regions (CDRs), interspersed with regions that are more conserved, termed framework regions (FR). VH and VL regions are 35 typically composed of three CDRs and four FRs, arranged from amino-terminus to carboxy-

terminus in the following order: FR1, CDR1, FR2, CDR2, FR3, CDR3, FR4. The hypervariable regions of the heavy and light chains form a binding domain that is capable of interacting with an antigen, whilst the constant region of an antibody may mediate binding of the immunoglobulin to host tissues or factors, including but not limited to various cells of the immune system (effector cells), Fc receptors and the first component (Clq) of the classical complement system.

Examples of antigen-binding fragments include Fab, Fab', F(ab)2, F(ab')2, F(ab)S, Fv (typically the VL and VH domains of a single arm of an antibody), single-chain Fv (scFv; see e.g.. Bird et al., Science 1988; 242:42S-426; and Huston et al. PNAS 1988; 85:5879-5883), dsFv, Fd (typically the VH and CHI domain), and dAb (typically a VH domain) fragments; VH, VL, VhH, and V-NAR domains; monovalent molecules comprising a single VH and a single VL chain; minibodies, diabodies, triabodies, tetrabodies, and kappa bodies (see, e.g., Ill et al. Protein Eng 1997;10:949-57); camel IgG; IgNAR; as well as one or more isolated CDRs or a functional paratope, where the isolated CDRs antigen-binding residues or polypeptides can be associated or linked together so as to form a functional antibody fragment. Various types of antibody fragments have been described or reviewed in, e.g., Holliger and Hudson, Nat Biotechnol 2005;2S:1126-1136; WO2005040219, and published U.S. Patent Applications 20050238646 and 20020161201.

The term "**antigen-binding fragment**" of an antibody refers to one or more fragments of an antibody that retain the ability to specifically bind to an antigen. It has been shown that the antigen-binding function of an antibody can be performed by fragments of a full-length antibody. Examples of binding fragments encompassed within the term "antigen-binding fragment" of an antibody include a Fab fragment, a $F(ab')_2$ fragment, a Fab' fragment, a Fd fragment, a Fv fragment, a ScFv fragment, a dAb fragment and an isolated complementarity determining region (CDR). Single chain antibodies such as scFv and heavy chain antibodies such as VHH and camel antibodies are also encompassed within the term "antigen-binding portion" of an antibody. These antibody fragments may be obtained using conventional techniques known to those of skill in the art, and the fragments may be screened for utility in the same manner as intact antibodies.

"**Fab fragments**", including "Fab" and "$Fab(ab')_2$" fragments, of an antibody are derived from said antibody by cleavage of the heavy chain in the hinge region on the N-terminal or C-terminal side of the hinge cysteine residues connecting the heavy chains of the antibody. A "**Fab**" fragment includes the variable and constant domains of the light chain and the variable domain and the first constant domain (CH1) of the heavy chain. "$F(ab')_2$" fragments comprise a pair of "Fab" fragments that are generally covalently linked by their

hinge cysteines. A Fab' is usually derived from a $F(ab')_2$ fragment by cleavage of the hinge disulfide bonds connecting the heavy chains in the $F(ab')_2$. Other chemical couplings than disulfide linkages of antibody fragments are also known in the art. A Fab fragment retains the ability of the parent antibody to bind to its antigen. $F(ab')_2$ fragments are capable of divalent
5 binding, whereas Fab and Fab' fragments can bind monovalently. Generally, Fab fragments lack the constant CH2 and CH3 domains, i.e. the Fc part, where interaction with the Fc receptors would occur. Thus, Fab fragments are in general devoid of effector functions. Fab fragments may be produced by methods known in the art, either by enzymatic cleavage of an antibody, e.g. using papain to obtain the Fab or pepsin to obtain the $F(ab')_2$, or Fab
10 fragments may be produced recombinantly using techniques that are well known to the person skilled in the art.

An "**Fv**" fragment is an antibody fragment that contains an antigen recognition and binding site, and generally comprises a dimer of one heavy chain variable domain and one light chain variable domain. The association between the heavy chain variable domain and
15 the light chain variable domain can be covalent in nature, for example in a single chain variable domain fragment (scFv). It is in this configuration that the three hypervariable regions of each variable domain interact to define an antigen-binding site/paratope on the surface of the VH-VL dimer. Collectively, the six hypervariable regions or a subset thereof confer antigen binding specificity to the antibody. However, even a single variable domain
20 comprising only three hypervariable regions specific for an antigen has the ability to recognize and bind antigen, although usually at a lower affinity than the entire binding site (Cai & Garen, *Proc. Natl. Acad. Sci. USA*, 93: 6280-6285, 1996). For example, naturally occurring camelid antibodies that only have a heavy chain variable domain (VHH) can bind antigen (Desmyter et al., *J. Biol. Chem.*, 277: 23645-23650, 2002; Bond et al., *J. Mol. Biol.*
25 2003; 332: 643-655).

"**Single-chain Fv**" or "**scFv**" antibody fragments comprise the VH and VL domains, where these domains are present in a single polypeptide chain. Generally, the Fv polypeptide further comprises a polypeptide linker between the VH and VL domains that enables the scFv to form the desired structure for antigen binding. For a review of scFv, see
30 Pluckthun, 1994, In: *The Pharmacology of Monoclonal Antibodies,* Vol. 113, Rosenburg and Moore eds. Springer-Verlag, New York, pp. 269-315.

The term "**diabodies**" refers to small antibody fragments with two antigen-binding sites, in which fragments comprise a heavy chain variable domain (VH) connected to a light chain variable domain (VL) in the same polypeptide chain (VH and VL) by way of a linker. By
35 using a linker that is too short to allow pairing between the two variable domains on the same

chain, the variable domains are forced to pair with complementary domains of another chain, creating two antigen-binding sites. Diabodies are described more fully, for example, in EP 404,097; WO 93/11161; and Hollinger et al., 1993, *Proc. Natl. Acad. Sci. USA,* 90:6444-6448.

5 The expression "**linear antibodies**" refers to antibodies as described in Zapata et al., 1995, *Protein Eng.,* 8(10):1057-1062. Briefly, these antibodies contain a pair of tandem Fd segments (VH-CH1-VH-CH1) that, together with complementary light chain polypeptides, form a pair of antigen binding regions. Linear antibodies can be bispecific or monospecific.

The term "**monobody**" as used herein, refers to an antigen binding molecule with a 10 heavy chain variable domain and no light chain variable domain. A monobody can bind to an antigen in the absence of light chains and typically has three hypervariable regions, for example CDRs designated CDRH1, CDRH2, and CDRH3. An IgG monobody has two heavy chain antigen binding molecules connected by a disulfide bond. The heavy chain variable domain comprises one or more hypervariable regions, preferably a CDRH3 or HVL-H3 15 region.

Antibody fragments may be obtained using conventional recombinant or protein engineering techniques. Antibody fragments of the invention may be made by truncation, e.g. by removal of one or more amino acid residues from the N and/or C-terminal ends of a polypeptide. Fragments may also be generated by one or more internal deletions.

20 An antibody of the invention may be a human antibody or a humanized antibody. The term "**human antibody**", as used herein, is intended to include antibodies having variable regions in which at least a portion of a framework region and/or at least a portion of a CDR region are derived from human germline immunoglobulin sequences. For example, a human antibody may have variable regions in which both the framework and CDR regions 25 are derived from human germline immunoglobulin sequences. Furthermore, if the antibody contains a constant region, the constant region is also derived from human germline immunoglobulin sequences. The human antibodies of the invention may include amino acid residues not encoded by human germline immunoglobulin sequences (*e.g.,* mutations introduced by random or site-specific mutagenesis *in vitro* or by somatic mutation *in vivo*).

30 Such a human antibody may be a human monoclonal antibody. Such a human monoclonal antibody may be produced by a hybridoma which includes a B cell obtained from a transgenic nonhuman animal, *e.g.,* a transgenic mouse, having a genome comprising a human heavy chain transgene and a light chain transgene fused to an immortalized cell.

Human antibodies may be isolated from sequence libraries built on selections of 35 human germline sequences, further diversified with natural and synthetic sequence diversity.

Human antibodies may be prepared by *in vitro* immunisation of human lymphocytes followed by transformation of the lymphocytes with Epstein-Barr virus.

The term "**human antibody derivative**" refers to any modified form of the human antibody, such as a conjugate of the antibody.

5 The term "**humanized antibody**", as used herein, refers to a human/non-human chimeric antibody that contains a sequence (CDR regions) derived from a non-human immunoglobulin. A humanized antibody is, thus, a human immunoglobulin (recipient antibody) in which at least one or more residues from a hyper-variable region of the recipient are replaced by residues from a hypervariable region of a non-human species (donor
10 antibody) for example from a mouse, rat, rabbit, or non-human primate, which have a desired property, such as specificity, affinity, and capacity. In some instances, FR residues of the human immunoglobulin are replaced by corresponding non-human residues. An example of such a modification is the introduction of one or more so-called back-mutations.

Furthermore, humanized antibodies may comprise residues that are not found in the
15 recipient antibody or in the donor antibody. These modifications are made to further refine antibody performance. In general, a humanized antibody will comprise at least one – typically two – variable domains, in which all or substantially all of the CDR regions correspond to those of a non-human immunoglobulin and in which all or substantially all of the FR residues are those of a human immunoglobulin sequence. The humanized antibody can, optionally,
20 also comprise at least a portion of an immunoglobulin constant region (Fc), typically that of a human immunoglobulin.

The term "**humanized antibody derivative**" refers to any modified form of the humanized antibody, such as a conjugate of the antibody and another agent or antibody.

The term "**chimeric antibody**", as used herein, refers to an antibody whose light
25 and heavy chain genes have been constructed, typically by genetic engineering, from immunoglobulin variable and constant region genes that originate from different species. For example, the variable segments of genes from a mouse monoclonal antibody may be joined to human constant segments.

The **fragment crystallizable region** ("**Fc region**"/"**Fc domain**") of an antibody is
30 the C-terminal region of an antibody, which comprises the constant CH2 and CH3 domains. The Fc domain may interact with cell surface receptors called Fc receptors, as well as some proteins of the complement system. The Fc region enables antibodies to interact with the immune system. In one aspect of the invention, antibodies may be engineered to include modifications within the Fc region, typically to alter one or more of its functional properties
35 (i.e. Fc effector functions), such as serum half-life, complement fixation, Fc-receptor binding,

protein stability and/or antigen-dependent cellular cytotoxicity, or lack thereof, among others. Furthermore, an antibody of the invention may be chemically modified (e.g., one or more chemical moieties can be attached to the antibody) or may be modified to alter its glycosylation, so as to alter one or more functional properties of the antibody. Preferably, a
5 modified Fc domain comprises one or more, and perhaps all of the following mutations that will result in decreased affinity to certain Fc receptors (L234A, L235E, and G237A) and in reduced C1q-mediated complement fixation (A330S and P331S), respectively (residue numbering according to the EU index). Antibodies having this type of Fc domain are sometimes referred to as “non-depleting” antibodies.

10 Antibodies without an Fc domain, such as e.g. therapeutic Fab fragments and other monovalent antibodies, tend to have a relatively short *in vivo* circulatory half life. In order to decrease the frequency of infusions with this type of therapeutic molecules, it may be desirable to conjugate such molecules with a “**half life extending moiety”**. Examples of half life extending moieties include: Biocompatible fatty acids and derivatives thereof, Hydroxy
15 Alkyl Starch (HAS) e.g. Hydroxy Ethyl Starch (HES), Poly Ethylen Glycol (PEG), Poly (Glyx-Sery)n (HAP), Hyaluronic acid (HA), Heparosan polymers (HEP), Phosphorylcholine-based polymers (PC polymer), Fleximers, Dextran, Poly-sialic acids (PSA), an Fc domain, Transferrin, Albumin, Elastin like peptides, XTEN polymers, Albumin binding peptides, a CTP peptide, and any combination thereof.

20 The isotype of an antibody of the invention may be IgG, such as IgG1, such as IgG2, such as IgG4. If desired, the class of an antibody may be “**switched**” by known techniques. For example, an antibody that was originally produced as an IgM molecule may be class switched to an IgG antibody. Class switching techniques also may be used to convert one IgG subclass to another, for example: from IgG1 to IgG2 or IgG4; from IgG2 to
25 IgG1 or IgG4; or from IgG4 to IgG1 or IgG2. Engineering of antibodies to generate constant region chimeric molecules, by combination of regions from different IgG subclasses, can also be performed.

In one embodiment, the hinge region of CH1 is modified such that the number of cysteine residues in the hinge region is altered, e.g., increased or decreased. This approach
30 is described further for instance in U.S. Patent No. 5,677,425 by Bodmer et al.

The constant region may further be modified to stabilize the antibody, e.g., to reduce the risk of a bivalent antibody separating into two monovalent VH-VL fragments. For example, in an IgG4 constant region, residue S241 may be mutated to a proline (P) residue to allow complete disulphide bridge formation at the hinge (see, e.g., Angal et al., Mol
35 Immunol. 199S; 30:105-8).

Antibodies or fragments thereof may also be defined in terms of their **complementarity determining regions (CDRs)**. The term "**complementarity determining region**" or "**hypervariable region**", when used herein, refers to the regions of an antibody in which amino acid residues involved in antigen binding are situated. The CDRs are generally
5 comprised of amino acid residues 24-34 (L1), 50-56 (L2) and 89-97 (L3) in the light-chain variable domain and 31-35 (H1), 50-65 (H2) and 95-102 (H3) in the heavy-chain variable domain; (Kabat *et al.* (1991) Sequences of Proteins of Immunological Interest, Fifth Edition, U.S. Department of Health and Human Services, NIH Publication No. 91-3242) and/or those residues from a "hypervariable loop" (residues 26-32 (L1), 50-52 (L2) and 91-96 (L3) in the
10 light-chain variable domain and 26-32 (H1), 53-55 (H2) and 96-101 (H3) in the heavy-chain variable domain; Chothia and Lesk, J. Mol. Biol 1987;196:901-917).

The term "**antigen**" **(Ag)** refers to the molecular entity used for immunization of an immunocompetent vertebrate to produce the antibody (Ab) that recognizes the Ag. Herein, Ag is termed more broadly and is generally intended to include target molecules that are
15 specifically recognized by the Ab, thus including fragments or mimics of the molecule used in the immunization process, or other process, e.g. phage display, used for generating the Ab.

The term "**epitope**", as used herein, is defined in the context of a molecular interaction between an "antigen binding polypeptide", such as an antibody (Ab), and its corresponding antigen (Ag). Generally, "epitope" refers to the area or region on an Ag to
20 which an Ab specifically binds, i.e. the area or region in physical contact with the Ab. Physical contact may be defined through various criteria (e.g. a distance cut-off of about 2-6Å, such as 3Å, such as 4 Å, such as 5Å; or solvent accessibility) for atoms in the Ab and Ag molecules. A protein epitope may comprise amino acid residues in the Ag that are directly involved in binding to a Ab (also called the immunodominant component of the epitope) and
25 other amino acid residues, which are not directly involved in binding, such as amino acid residues of the Ag which are effectively blocked by the Ab, i.e. amino acid residues within the "solvent-excluded surface" and/or the "footprint" of the Ab.

The epitope for a given Ab/Ag pair can be described and characterized at different levels of detail using a variety of experimental and computational epitope mapping methods.
30 The experimental methods include mutagenesis, X-ray crystallography, Nuclear Magnetic Resonance (NMR) spectroscopy, Hydrogen deuterium eXchange Mass Spectrometry (HX-MS) and various competition binding methods as well as other methods that are known in the art. As each method relies on a unique principle, the description of an epitope is intimately linked to the method by which it has been determined. Thus, depending on the epitope
35 mapping method employed, the epitope for a given Ab/Ag pair may be described differently.

At its most detailed level, the epitope for the interaction between the Ag and the Ab can be described by the spatial coordinates defining the atomic contacts present in the Ag-Ab interaction, as well as information about their relative contributions to the binding thermodynamics. At a less detailed level, the epitope can be characterized by the spatial coordinates defining the atomic contacts between the Ag and Ab. At an even less detailed level the epitope can be characterized by the amino acid residues that it comprises as defined by a specific criteria such as the distance between or solvent accessibility of atoms in the Ab-Ag complex. At a further less detailed level the epitope can be characterized through function, e.g. by competition binding with other Abs. The epitope can also be defined more generically as comprising amino acid residues for which substitution by another amino acid will alter the characteristics of the interaction between the Ab and Ag.

Epitopes described at the amino acid level, e.g. determined from an X-ray structure, are said to be identical if they contain the same set of amino acid residues. Epitopes are said to overlap if at least one amino acid is shared by the epitopes. Epitopes are said to be separate (unique) if no amino acid residue is shared by the epitopes.

The definition of the term “**paratope**” is derived from the above definition of “epitope” by reversing the perspective. Thus, the term “paratope” refers to the area or region on the Ab to which an Ag specifically binds, i.e. with which it makes physical contact to the Ag. Thus, a paratope may be defined by using the definition for epitope and applying it to the antibody as opposed to the antigen.

Antibodies that bind to the same antigen can be characterised with respect to their ability to bind to their common antigen simultaneously and may be subjected to “**competition binding**”/“**binning**”. In the present context, the term “binning” refers to a method of grouping antibodies that bind to the same antigen. “Binning” of antibodies may be based on competition binding of two antibodies to their common antigen in assays based on standard techniques such as surface plasmon resonance (SPR), ELISA or flow cytometry.

A “**bin**” can be viewed as an epitope defined using a reference antibody. If a second antibody is unable to bind to the antigen at the same time as the reference antibody, the second antibody is said to belong to the same “bin” as the reference antibody. In this case the reference and the second antibody are competing for binding to the antigen, thus the pair of antibodies is termed “competing antibodies”. If a second antibody is capable of binding to the antigen at the same time as the reference antibody, the second antibody is said to belong to a separate “bin”. In this case the reference and the second antibody are not competing for binding to the antigen, thus the pair of antibodies is termed “non-competing antibodies”.

Antibody “binning” does not provide direct information about the epitope. Competing antibodies, i.e. antibodies belonging to the same “bin” may have identical epitopes, overlapping epitopes or even separate epitopes. The latter is the case if the reference antibody bound to its epitope on the antigen takes up the space required for the second antibody to contact its epitope on the antigen (“steric hindrance”). Non-competing antibodies generally have separate epitopes.

“Reduction/Inhibition of the interaction of OSCAR with collagen” or “competing with collagen for binding to OSCAR” means the ability of an antibody, or a fragment thereof, to reduce in a dose dependent manner, the binding of collagen or fragments thereof, to OSCAR. A method to measure this can be, but is not limited to, surface plasmon resonance (SPR) analysis performed on a biosensor instrument, e.g. Biacore instruments as described in example 13 (50 nM OSCAR is used as an analyte and mixed with 0-10 µg/ml prior to injection into e.g. a Biocore T 100 instrument (GE healthcare) Human collagen I and collagen II are immobilized on e.g. a Biacore CM3 sensor chip (GE Helathcare)) OSCAR Antibodies according to the invention having the ability to significantly reduce OSCAR:collagen binding are those that can reduce OSCAR:collagen binding by at least 15%, preferably at least 20%, preferably at least 25%, preferably at least 30%, preferably at least 35%, preferably at least 40%, preferably at least 45%, preferably at least 50%, preferably at least 55%, preferably at least 60%, preferably at least 65%, preferably at least 70%, preferably at least 75%, preferably at least 80%, preferably at least 85%, preferably at least 90%, preferably at least 95% compared to e.g. a control antibody.

Reduction of collagen induced cytokine secretion means the ability of an antibody, or a fragment thereof, to reduce in a dose dependent manner collagen induced cytokine secretion. Collagen, such as collagen I, can induce secretion of various cytokines (e.g. IL-8, TNF-α, etc.) by various cell types (e.g. synovial fluid mononuclear cells). Collagen may furthermore induce maturation of dendritic cells (DCs). Reduction of collagen induced cytokine secretion can e.g. be measured in the following way: Collagen I and collagen II are immobilized on a surface of essentially neutral charge (e.g. Costar #3361). RA synovial fluid cells (e.g. $3X10^5$/well) are preincubated with the anti OSCAR mAbs or control mAbs prior to be transferred onto the collagen coated plate. Cytokines are measured by ELISA. OSCAR antibodies according to the invention having the ability to reduce collagen induced cytokine secretion/DC cell maturation are those that can reduce cytokine secretion/DC cell maturation by at least 20%, preferably at least 25%, preferably at least 30%, preferably at least 35%,

preferably at least 40%, preferably at least 45%, preferably at least 50%, preferably at least 55%, preferably at least 60%, preferably at least 65%, preferably at least 70%, preferably at least 75%, preferably at least 80%, preferably at least 85%, preferably at least 90%, preferably at least 95% compared to e.g. a control antibody.

5

Reduction of TRAP positive (TRAP$^+$) osteoclast formation means the ability of an antibody, or a fragment thereof, to reduce in a dose dependent manner TRAP$^+$osteoclast formation. OSCAR antibodies according to the present invention may have the ability to reduce TRAP$^+$ cell numbers in normal monocytes – and thereby reducing osteoclast
10 formation. Reduction of TRAP$^+$ osteoclast formation can e.g. be detected using the following assay: 60,000 monocytes/well are cultured with recombinant human M-CSF (25 ng/ml) and soluble RANKL (100 ng/ml). The cells are cultured on uncoated plastic. After ended incubation (day 9-12), the cells are stained for TRAP, e.g. using a leukocyte acid phosphatase kit. Compared to a control antibody, OSCAR antibodies according to the
15 present invention may thus reduce TRAP$^+$5 cell numbers in normal monocytes by at least 20%, preferably at least 25%, preferably at least 30%, preferably at least 35%, preferably at least 40%, preferably at least 45%, preferably at least 50%, preferably at least 55%, preferably at least 60%, preferably at least 65%, preferably at least 70%, preferably at least 75%, preferably at least 80%, preferably at least 85%, preferably at least 90%, preferably at
20 least 95%.

Reduction of TRAP5b secretion by osteoclasts means the ability of an antibody, or a fragment thereof, to reduce in a dose dependent manner TRAP5b secretion by osteoclasts. OSCAR antibodies according to the present invention may have the ability to reduce
25 TRAP5b secretion by osteoclasts, wherein said osteoclasts are derived from normal monocytes. Reduction of TRAP5b secretion by osteoclasts can e.g. be detected using the following assay: 60,000 monocytes/well are cultured with recombinant human M-CSF (25 ng/ml) and soluble RANKL (100 ng/ml). The cells are cultured on uncoated plastic. After ended incubation (day 9-12), the supernatants from cell cultures are collected for ELISA
30 measurements of TRAP5b. Compared to a control antibody, OSCAR antibodies according to the present invention may thus reduce TRAP5b secretion by osteoclasts derived from normal monocytes by at least 20%, preferably at least 25%, preferably at least 30%, preferably at least 35%, preferably at least 40%, preferably at least 45%, preferably at least 50%, preferably at least 55%, preferably at least 60%, preferably at least 65%, preferably at least

70%, preferably at least 75%, preferably at least 80%, preferably at least 85%, preferably at least 90%, preferably at least 95%.

Reduction of bone resorption from osteologic discs and/or bone slices means the
5 ability of an antibody, or a fragment thereof, to reduce in a dose dependent manner bone resorption from osteologic discs and/or bone slices. Reduction of bone resorption can e.g. be detected using the following assay: 60,000 monocytes/well are cultured with recombinant human M-CSF (25 ng/ml) and soluble RANKL (100 ng/ml). The cells are cultured on osteologic dics or bone slices. After ended incubation (day 9-12), bone resorption can be
10 detected in one or more of the following way: (i) image analysis of erosion on osteologic discs /bone slides by e.g. Immunospot, and/or (ii) the supernatants are collected from the cell culture on bone slides and degraded collagen fragments are detected using e.g. CTX-1 ELISA. Compared to a control antibody, OSCAR antibodies according to the present invention may reduce bone resorption from osteologic discs and/or bone by at least 20%,
15 preferably at least 25%, preferably at least 30%, preferably at least 35%, preferably at least 40%, preferably at least 45%, preferably at least 50%, preferably at least 55%, preferably at least 60%, preferably at least 65%, preferably at least 70%, preferably at least 75%, preferably at least 80%, preferably at least 85%, preferably at least 90%, preferably at least 95%.

20

The terms “**immunoreacts”** or “immunoreacting”, as used herein, means any binding of an antibody to its epitope with a dissociation constant K_d lower than 10^{-4} M. The terms “immunoreacts” or “immunoreacting” are used where appropriate inter-changeably with the term "specifically bind".

25 The term **“affinity”**, as used herein, means the strength of the binding of an antibody to an epitope. The affinity of an antibody is measured by the dissociation constant K_d, defined as [Ab] x [Ag] / [Ab-Ag] where [Ab-Ag] is the molar concentration of the antibody-antigen complex, [Ab] is the molar concentration of the unbound antibody and [Ag] is the molar concentration of the unbound antigen. The affinity constant K_a is defined by $1/K_d$.
30 Preferred methods for determining antibody specificity and affinity by competitive inhibition can be found in Harlow, et al., Antibodies: A Laboratory Manual, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., 1988), Colligan et al., eds., Current Protocols in Immunology, Greene Publishing Assoc. and Wiley Interscience, N.Y., (1992, 1993), and Muller, Meth. Enzymol. 92:589-601 (1983).

An **“antagonist”** is a compound that does not provoke a biological response upon binding to a receptor or a ligand, but blocks or dampens agonist-mediated responses. On the contrary, an “agonist” is a compound that induces a response upon binding to a receptor or ligand. Upon binding to a target, an antagonist will reduce the cell response induced by an 5 agonist, such as e.g. a natural ligand or a compound that functionally resembles the effects of the natural ligand.

In connection with the present invention, the phrase “OSCAR induced effects” as used herein may refer to at least one of an inflammatory response or differentiation of OC. An inflammatory response can include but is not limited to activation of a cell of the immune 10 system (e.g., T and B lymphocytes; polymorphonuclear leukocytes or granulocytes, such as neutrophils; monocytes, dendritic cells, macrophages, and NK cells), recruitment of a cell of the immune system, or release of a cell-derived soluble pro-inflammatory mediator, such as for example a cytokine or chemokine. Differentiation of OC can include, but is not limited to, expression of cellular markers for OC differentiation by monocytes-derived precursors or 15 bone marrow precursors, increased cell fusion, increased tartrate-resistant acid phosphatase (TRAP) activity, increased cathepsin K expression, increased beta 3 integrin expression, multinucleation, and bone resorption. Consequently, a ligand can be said to antagonize OSCAR induced effects, for example, if it mediates at least about 20% inhibition, preferably at least about 30% inhibition, more preferably at least about 40% inhibition, more preferably 20 at least about 50% inhibition, and most preferably at least about 75% inhibition of IL-8 release from dendritic cells (DCc) induced by Coll (example 8) at antibody concentrations below 10 nM.

A **“receptor”** is a protein molecule, embedded in either the plasma membrane (e.g. a transmembrane protein) or present in the cytoplasm of a cell, to which one or more specific 25 signaling molecules may attach. A molecule which specifically binds (attaches) to a receptor is called a “ligand”, and may be an antibody, a peptide or other small molecule, such as a neurotransmitter, a hormone, a pharmaceutical drug, or a toxin. Ligand binding generally stabilizes a certain receptor conformation. This is often associated with gain of or loss of receptor activity, often leading to a cellular response. However, some ligands (e.g. 30 antagonists) merely block receptors without inducing any response. Ligand-induced changes in receptors result in cellular changes which constitute the biological activity of the ligands.

Production of antibodies: Monoclonal antibodies are typically made by fusing myeloma cells with the spleen cells from a mouse that has been immunized with the desired 35 antigen. Human antibodies can be obtained from transgenic animals (e.g. mice) encoding

human antibodies. Alternatively, recombinant monoclonal antibodies can be made and identified involving technologies, referred to as repertoire cloning or phage display/yeast display. Recombinant antibody identification involves the use of viruses or yeast to display antibodies for selection, rather than the use of e.g. mice.

5 Antibodies may be produced by means of recombinant techniques. The DNA sequences encoding the antibody are usually inserted into a recombinant vector. The vector is preferably an expression vector in which the DNA sequence is operably linked to a promoter capable of directing the transcription of a cloned antibody gene or antibody cDNA in the desired host cell.

10 After the cells have taken up the DNA, they are grown in an appropriate growth medium, typically from a few days to a few weeks. The host cell may be any cell, which is capable of producing the antibody and includes bacteria, yeast, other fungi and higher eucaryotic cells. Examples of mammalian cell lines for use in the present invention are COS-1, baby hamster kidney (BHK) and HEK293. A preferred BHK cell line is the tk- ts13 BHK cell
15 line that may be referred to as BHK 570 cells. In addition, a number of other cell lines, or their derivatives may be used within the present invention, including Rat Hep I, Rat Hep II, TCMK, NCTC 1469, CHO, and DUKX cells.

The transformed or transfected host cell described above is then cultured in a suitable nutrient medium under conditions permitting expression of the antibody. The
20 antibody may be subsequently recovered from the culture by conventional procedures including separating the host cells from the medium by centrifugation or filtration, precipitating the proteinaceous components of the supernatant or its filtrate (for example, by means of a salt, e.g. ammonium sulphate), purification by a variety of chromatographic procedures, e.g. ion exchange chromatography, gelfiltration chromatography, affinity
25 chromatography, or the like, dependent on the type of polypeptide in question.

Transgenic animal technology may be employed to produce the antibody of the invention. It is preferred to produce the antibodies in the mammary glands of a host female mammal, preferably in sheeps, goats or cattle. Production in transgenic plants may also be employed. Expression may be directed to a particular organ, such as a tuber.

30

The present invention further includes **pharmaceutical compositions**/formulations comprising antibodies according to the invention and optionally comprising a pharmaceutically acceptable carrier. The pharmaceutical composition according to the invention may be in the form of an aqueous formulation or a dried formulation that is
35 reconstituted in water/an aqueous buffer prior to administration. Pharmaceutical

compositions comprising compounds according to the invention may be supplied as a kit comprising a container that comprises the products according to the invention. Therapeutic polypeptides can be provided in the form of an injectable solution for single or multiple doses, or as a sterile powder that will be reconstituted before injection. Pharmaceutical compositions comprising compounds according to the invention are suitable for subcutaneous and/or IV administration.

LIST OF EMBODIMENTS

The present invention is exemplified in the following non-limiting embodiments (it is understood that all embodiments can be combined):

1. An antibody that binds to human OSCAR and antagonizes OSCAR induced effects, wherein said antibody competes with antibody 7F1A1 for binding to OSCAR, wherein the amino acid sequence of the 7F1A1 heavy chain is as set forth in SEQ ID NO 6 and the amino acid sequence of the 7F1A1 light chain is as set forth in SEQ ID NO 5.

2. An antibody according to the invention, wherein said antibody binds to the same epitope, or at least a portion of the same epitope, as the 7F1A1 antibody.

3. An antibody that binds to human OSCAR and antagonizes OSCAR induced effects, wherein said antibody competes with antibody 7F21A1 for binding to OSCAR, wherein the amino acid sequence of the 7F21A1 heavy chain is as set forth in SEQ ID NO 16 and the amino acid sequence of the 7F21A1 light chain is as set forth in SEQ ID NO 15.

4. An antibody according to the invention, wherein said antibody binds to the same epitope, or at least a portion of the same epitope, as the 7F21A1 antibody.

5. An antibody that binds to human OSCAR and antagonizes OSCAR induced effects, wherein said antibody competes with antibody 3F7A9 for binding to OSCAR, wherein the amino acid sequence of the 3F7A9 antibody heavy chain is as set forth in SEQ ID NO 20 and the amino acid sequence of the 3F7A9 light chain is as set forth in SEQ ID NO 19.

6. An antibody according to the invention, wherein said antibody binds to the same epitope, or at least a portion of the same epitope, as the 7F7A9 antibody.

7. An antibody according to the invention, wherein said antibody is not an R-phycoerythrin conjugated antibody.

8. An antibody according to the invention, wherein said antibody is not an 11.1CN5 antibody.

9. An antibody comprising the three CDR sequences as set forth in SEQ ID NO 6 and three CDR sequences as set forth in SEQ ID NO 5. Preferably, said antibody comprises at least one CDR (e.g. CDR3) from SEQ ID NO 6 and at least one CDR (e.g. CDR3) from SEQ ID NO 5. One or two amino acid alternations (substitutions and/or deletions and/or insertions) may be introduced in one, two, three, four, five or six of said CDR sequences. Alternatively, said antibody comprises the entire VH/VL sequences of SEQ ID NO 5 and SEQ ID NO 6.

10. An antibody comprising the three CDR sequences as set forth in SEQ ID NO 16 and the three CDR sequences as set forth in SEQ ID NO 15. Preferably, said antibody comprises at least one CDR (e.g. CDR3) from SEQ ID NO 16 and at least one CDR (e.g. CDR3) from SEQ ID NO 15. One or two amino acid alternations (substitutions and/or deletions and/or insertions) may be introduced in one, two, three, four, five or six of said CDR sequences. Alternatively, said antibody comprises the entire VH/VL sequences of SEQ ID NO 15 and SEQ ID NO 16.

11. An antibody comprising the three CDR sequences as set forth in SEQ ID NO 19 and the three CDR sequences as set forth in SEQ ID NO 20. Preferably, said antibody comprises at least one CDR (e.g. CDR3) from SEQ ID NO 19 and at least one CDR (e.g. CDR3) from SEQ ID NO 20. One or two amino acid alternations (substitutions and/or deletions and/or insertions) may be introduced in one, two, three, four, five or six of said CDR sequences. Alternatively, said antibody comprises the entire VH/VL sequences of SEQ ID NO 19 and SEQ ID NO 20.

12. An antibody according to the invention, wherein said antibody reduces collagen I and/or collagen II induced secretion of TNF-α and/or IL-8 in immature dendritic cells (DC).

13. An antibody according to the invention, wherein said antibody reduces collagen I induced secretion of IL-8 and/or TNF-alpha from synovial fluid mononuclear cells from rheumatoid arthritis (RA) patients.

14. A pharmaceutical composition comprising an antibody according to the invention.

15. A pharmaceutical composition according to the invention, wherein no sodium azide has been added to said composition.

16. Use of an antibody according to the invention, or a pharmaceutical composition according to the invention, for treating an autoimmune inflammatory disease.

17. Use of an antibody according to the invention, or a pharmaceutical composition according to the invention, for treating RA.

18. Use of an antibody according to the invention, or a pharmaceutical composition according to the invention, for treating a bone resorption related disorder.

19. A method for inducing collagen dependent maturation of monocyte-derived Dendritic Cells (moDCs), wherein said method comprises incubating moDCs on a surface of essentially neutral charge in the presence of collagen.

20. A DNA molecule encoding an antibody according to the invention.

21. An expression vector comprising a DNA molecule according to the invention.

22. A host cell comprising an expression vector according to the invention and/or a DNA molecule according to the invention.

23. A method for making an antibody according to the invention, wherein said method comprises incubating a host cell according to the invention under conditions

appropriate for expression of said antibody and optionally subsequently isolating said antibody.

24. Use of an antibody according to the invention, or a pharmaceutical composition according to the invention as a medicine.

25. A method of treatment comprising administering to a person in need thereof an appropriate amount of an antibody according to the invention for treatment of an autoimmune inflammatory disease, such as e.g. RA.

26. A method of treatment comprising administering to a person in need thereof an appropriate amount of an antibody according to the invention for treatment of a bone resorption related disorder, such as e.g. osteoarthritis, osteoporosis, psoriatic arthritis.

27. A method for inducing collagen dependent maturation of moDCs, wherein said method comprises incubating moDCs on a virgin polystyrene surface in the presence of collagen.

28. A method for inducing collagen dependent maturation of moDCs according to the invention, wherein collagen is selected from the group consisting of collagen I, collagen II, and collagen III.

29. Use of a method for inducing collagen dependent maturation of moDCs according to the invention for screening OSCAR antibodies for their ability to reduce maturation of moDcs.

30. An OSCAR variant comprising a C83A substitution.

31. Use of the OSCAR variant according to the invention for resolving OSCAR crystal structures.

32. Use of the OSCAR variant according to the invention for resolving the crystal structure of OSCAR bound to a ligand.

EXAMPLES

Example 1

Production and selection of OSCAR antibodies

5 Immunization and fusion

In order to generate mouse monoclonal antibodies (mAb) BALB/c mice were immunized with the recombinant extracellular domain of human OSCAR (OSCAR-ECD (SEQ ID NO 2). Fully human mAbs were generated by immunization of transgenic mice (HuMab mice developed by Medarex Inc.) with OSCAR-ECD (SEQ ID NO 2). The HuMab mice have 10 disabled murine IgG loci, and inserted loci encoding human IgG.

Mice were immunized subcutaneously. For the first immunization, 20µg of antigen was mixed with complete Freunds adjuvant. In subsequent immunizations incomplete Freund's adjuvant was used with the same amount of antigen. Ten days after the last immunization, eye-blood from the mice was analyzed by ELISA for OSCAR specific antibodies. Mice with positive 15 titers were boosted intravenously with 10µg antigen in PBS, and sacrificed after 3 days. The spleens were aseptically removed and dispersed into a single cell suspension. Fusion of spleen cells and myeloma cells was performed using either the PEG-method or electrofusion. Hybridomas secreting specific antibodies were selected using specific binding assays as described below.

20

Binding assays: ELISA

Immunoplates (Maxisorb, Nunc) were coated with 2µg/ml OSCAR-ECD. Hybridoma culture supernatants were added to the plates. Detection was carried out with a HRP-conjugated polyclonal antibody (pAb) specific to murine or human antibodies.

25

Binding assays: flow cytometry (FACS)

Binding of antibodies to OSCAR was first examined in HEK293 cells transfected to express extracellular OSCAR. By using transfected cells it was possible to counter-screen for un-specific binding to the parental mock-transfected HEK293 cell line. The screening for 30 OSCAR specific antibodies was done in sera, culture supernatants and in purified mAbs. Antibodies which selectively bound to the HEK-OSCAR cells were further tested for binding to primary monocytes from human PBMC expressing endogenous OSCAR.

This was done to ensure selection of antibodies which recognise OSCAR under physiological conditions.

35

Establishment of the HEK-OSCAR cell line

Full length human OSCAR (hOSCAR) cDNA (GenBank #AF391162) was cloned into the *Nhe*I-*Bam*HI sites of the mammalian expression vector pIRESneo2 (Clontech, #6938-1) and used for transfection of HEK293 cells. Transfected clones were selected for drug resistance (Geneticin, Invitrogen #10131-035). Expression of OSCAR was analysed by immunoblotting using anti-hOSCAR pAb (R&D, #AF2004). Cell surface expression of OSCAR was confirmed by FACS using the anti-OSCAR-PE (phycoerythrin) mAb clone 11.1CN5 (Beckman Coulter, #PN A24987). The HEK-hOSCAR clone 2-2D9 with the highest expression of OSCAR was selected for screening of anti-OSCAR mAbs. The geneticin-resistant pool of HEK293 cells transfected with pIRESneo2 (mock-transfectants) served as negative control.

Purification of primary human mononuclear cells

Buffy coats from healthy individuals were obtained from the University Hospital of Copenhagen. The cells were diluted in RPMI1640 and 25 ml put on top of 12,5ml Ficoll-Paque Plus reagents (Amersham Biosciences) and spun down at 950xg for 30 min at room temperature. The peripheral blood mononuclear cells (PBMC) isolated by density gradient centrifugation were recovered from the interphase and thoroughly washed several times in RPMI1640 medium, spun and re-suspended either in a washing buffer (1x PBS (-Ca^{2+}/-Mg^{2+}) + 1% BSA) for immediate use or in a cryo-medium (RPMI1640 supplemented with 20% FBS and 10% DMSO) for cryopreservation. Both freshly isolated and freeze-thawed cells were equally suitable for immunostaining followed by FACS analysis.

Immunostaining and FACS analysis

Cell number and viability were determined using a Cedex cell counter. The cells were diluted in washing buffer (1x PBS (-Ca^{2+}/-Mg^{2+}) + 5% FBS + 0.1% Na-azide) to 10^7cells/90µl. In the case of monocytes, the cells were further blocked by adding 10µl of a hFcR blocker (Miltenyi, # 130-059-901) and incubated for 10 minutes at 4°C. 50µl of cells (50.000cells/well) were seeded into 96 well round-bottomed microtiter plates. Hybridoma supernatants or purified antibodies (in serial dilutions) were added to the cells at 50µl/well. The isotype-matched purified irrelevant human mAb or mouse mAb served as negative controls (mouse IgG1, BD, #553452; mouse IgG2a, BD, #557353; mouse IgG2b, BD, #557352). The cells were incubated in the dark for 60 minutes at 4°C, washed twice and stained with the secondary antibodies (goat anti-human IgG –PE, Jackson ImmunoResearch, #109-116-170 or donkey anti-mouse IgG-APC (allophycocyanin), Jackson ImmunoResearch, #715-136-150), diluted 1:500, for 30 minutes in the dark at 4°C. Cells were washed in PBS, re-suspended in fixation

buffer (1 x PBS (-Ca^{2+} /-Mg^{2+}), pH7.2 + 1% formalin (B&B, #LAB96751.0500) and analysed by FACS flow cytometer. EC_{50} values were calculated using a GraphPad Prism 5 software.

Results and conclusions

5 The majority of the mAbs which specifically bound to HEK-OSCAR cells were also capable of binding to primary human monocytes. The strongest binders were selected for further characterisation in functional assays. EC_{50} values of the selected mAbs binding to human monocytes are shown in Table 1.

10 **Table 1:** Binding of anti-OSCAR mAbs to human monocytes

mAb	EC_{50}, nM (Donor #1)	EC50, nM (Donor #2)
7F1A1	1.21	0.12
7F6A1	3.48	0.14
7F14A1	2.47	0.55
7F21A1	9.48	0.01
7F10A1	1.2	0.12
13F5A5	4.02	2.18
13F56A6	1.54	2.39
3F7A9	1.79	0.14
4F3B	0.66	0.32
1F7C7	4.63	3.68

Example 2

Determination of the antigen binding affinity

Affinities of the anti-OSCAR mAbs towards OSCAR-ECD (SEQ ID NO 2) were measured by
15 SPR using a Biacore T100 instrument (GE Healthcare). These studies were performed using either a direct binding procedure, with the respective mAb covalently coupled via free amine groups to the carboxy methylated dextrane membrane (CM5) on the sensor chip surface, or a capture method where the individual mAbs were captured on immobilized anti-mouse or anti-human pAbs prior to injection of OSACAR-ECD. OSCAR-ECD was injected in various
20 concentrations, followed by a dissociation period with constant buffer flow over the sensor chip surface. The kinetic parameters (k_a, k_d and K_D) for the interaction were calculated using a 1:1 interaction Langmuir fitting model.

Results

The measured affinities were all in the nM range and comparable to that of the mAb 11.1CN5 which was shown to interfere with the OSCAR-ColI interaction. The affinity of OSCAR-ECD to ColI has been determined as 255nM by SPR analysis. The sub-μM affinity of this
5 interaction is within the range of the affinities reported for ColI interaction with other members of the LRC family, including LAIR-1/-2 and GPVI. Thus, the affinities of the mAbs to OSCAR are approximately 20-360 folds higher than the affinity of the OSCAR-ECD/ColI complex formation. Results are shown in Table 2.

10 **Table 2:** Affinities of OSCAR mAbs

OSCAR mAbs	**K_D (nM)**
3F7A9	9.1
4F3B	1.7
7F1A1	2.7
7F6A1	2.7
7F10A1	18
7F21A1	18
7F14A1	4.5
13F5A5	0.7
13F56A6	3.0
16F56	0.3
16F119	0.5
11.1CN5	6.4

Example 3

Cloning and sequencing of the anti-OSCAR mAb cDNAs

The cDNAs of the light and heavy chains were cloned by RT-PCR using total RNA extracted
15 from 10^7 cells of each anti-hOSCAR hybridomas 4F3B, 7F1A, 7F6A, 7F9A, 7F10A, 7F14A, 7F21A, and 3F7A9. Reverse transcription was performed using oligo-dT primer. Antibody cDNAs were amplified using specific primers. The amplified products were cloned into the pMD19-T vector (Takara, China) and sequenced. The cDNAs were then sub-cloned into a vector for expression in mammalian cells. The recombinant antibody were produced in
20 HEK2936E cells and purified.

Amino acid sequences of the selected mAbs are listed below.

7F1A1 light chain and heavy chain sequences (CDR sequences shown in bold/underline and VL/VH sequences are shown in capital letters):

7F1A1 Light Chain (SEQ ID NO 5)

mklpvrllvllwltdarcDIQMTQSPASLSVSVGETVTITC**RASEKIYSNLA**WYQQKQGKSPQLLVY**A**
ATNLADGVPSRFSGSGSGTQFSLKINSLQPEDFGSYYC**QHFWGIPWT**FGGGTKLEIKRada
aptvsifppsseqltsggasvvcflnnfypkdinvkwkidgserqngvlnswtdqdskdstysmsstltltkdeyerhnsytceath
ktstspivksfnrnec

7F1A1 Heavy Chain (SEQ ID NO 6)

mgwswvflfllsvtagvhsQVQLQQSGAELVRPGTSVKVSCKASGYAFT**NYMIE**WVKKRPGQGLE
WIG**VINPGSGGTNYNEKFEN**KATLTADKSSSTAYMQLSSLTSDDSAVYFCAR**SLDFDGAWF**
AYWGQGTLVTVSAakttppsvyplapgsaaqtnsmvtlgclvkgyfpepvtvtwnsgslssgvhtfpavlqsdlytlsssvt
vpsstwpsetvtcnvahpasstkvdkkivprdc

7F6A1 light chain and heavy chain sequences (CDR sequences shown in bold/underline and VL/VH sequences are shown in capital letters):

7F6A1 Light Chain (SEQ ID NO 7)

mdlqvqiisfllisasvimsrgENVLTQSPAIMSASPGEKVTMTC**SVSSSISYMH**WYRQKSFTSPQLWI
Y**DTSKLAS**GVPGRFSGSGSGISYSLTISSMEAEDVATYYC**FQGSEYPLT**FGAGTKLELKRad
aaptvsifppsseqltsggasvvcflnnfypkdinvkwkidgserqngvlnswtdqdskdstysmsstltltkdeyerhnsytceat
hktstspivksfnrnec

7F6A1 Heavy chain (SEQ ID NO 8)

mdfglsliflvltlkgvqcDVKLVESGGGLVKPGGSLKLSCAASGFTFS**SYVMS**WVRQTPEKRLEWV
A**TISSGGSYTYYPDSVKG**RFTISRDNAKNTLYLQMSSLKSEDTAMYYCTR**EGGNSPDY**WG
QGTTLTVSSakttppsvyplapgsaaqtnsmvtlgclvkgyfpepvtvtwnsgslssgvhtfpavlqsdlytlsssvtvpsstw
psetvtcnvahpasstkvdkkivprdc

7F9A1 light chain and heavy chain sequences (CDR sequences shown in bold/underline and VL/VH sequences are shown in capital letters):

7F9A1 Light Chain (SEQ ID NO 9)

metdtlllwvlllwvpgstgDIVLTQSPASLAVSLGQRATISC**RASQSVSTSNSSYMH**WCQQKPGQPP
KLLIK**YASNLES**GVPARFSGSGSGTDFTLNIHPVEEEDTATYYC**QHSWEIPFT**FGGGTKLEIK
Radaaptvsifppsseqltsggasvvcflnnfypkdinvkwkidgserqngvlnswtdqdskdstysmsstltltkdeyerhnsyt
ceathktstspivksfnrnec

7F9A1 Heavy Chain (SEQ ID NO 10)

mewswvfifllsgtagvlsEVQLQQFGAELVKPGASVKISCKASGYTFT**DYNMD**WVKQSHGKSLEW
IG**DINPNYDSTTYNQKFKG**KATLTVDKSSSTAYMELRSLTSEDTAVYYCAR**AYGNYDWWYF**
DVWGAGTTVTVSSakttppsvyplapgsaaqtnsmvtlgclvkgyfpepvtvtwnsgslssgvhtfpavlqsdlytlsssvt
vpsstwpsetvtcnvahpasstkvdkkivprdc

7F10A1 light chain and heavy chain sequences (CDR sequences shown in bold/underline and VL/VH sequences are shown in capital letters):

7F10A1 Light Chain (SEQ ID NO 11)

mdfqvqifsfllisasVIMSRGQILLTQSPAIMSASPGEKVTMTC**SASSSVSYMH**WYQQKPGSSPKP
WIY**DTSNLAS**GFPARFSGSGSGTSYSLIISSMEAEDAATYYC**HQRSSYPYT**FGGGTKLEIKR
adaaptvsifppsseqltsggasvvcflnnfypkdinvkwkidgserqngvlnswtdqdskdstysmsstltltkdeyerhnsytce
athktstspivksfnrnec

7F10A1 Heavy Chain (SEQ ID NO 12)

mgwswvflfilsgtagvhsQVQLQQSGAELARPGASVKLSCKASGYTFT**DNYIN**WVKQRTGQGLE
WIG**EIYPGSGNTYYNEKFKG**KATLTADKSSSTAYMQLSSLTSEDSAVYFCAR**SANRYDRGD**
FDYWGQGTPLTVSSakttppsvyplapgsaaqtnsmvtlgclvkgyfpepvtvtwnsgslssgvhtfpavlqsdlytlsss
vtvpsstwpsetvtcnvahpasstkvdkkivprdc

7F14A1 light chain and heavy chain sequences (CDR sequences shown in bold/underline and VL/VH sequences are shown in capital letters):

7F14A1 Light Chain (SEQ ID NO 13)

mdfqvqiisfllisasviisrgQIVLTQSPAIMSASPGEKVTMTC**SASSSVNYMH**WYQQKSGTSPKRWI Y**DTSNLAS**GVPTRFSGSGSGTSYSLTISSMEAEDAATYYC**QQWSSDPLT**FGAGTKLELKRa daaptvsifppsseqltsggasvvcflnnfypkdinvkwkidgserqngvlnswtdqdskdstysmsstltltkdeyerhnsytcea thktstspivksfnrnec

7F14A1 Heavy Chain (SEQ ID NO 14)

*rlwlvlgllfclvafpscvlsQVQLKESGPGLVAPSQSLSITCSVSGFSLT**SYGVH**WVRQPPGKGLEW LG**VMWAGGSTNYNSALMS**RLSISKDNSKSQVFLKMNSLQTDDSAMYYCAL**QKGYGNYFD Y**WGQGTTLTVSSakttppsvyplapgsaaqtnsmvtlgclvkgyfpepvtvtwnsgslssgvhtfpavlqsdlytlsssvtv psstwpsetvtcnvahpasstkvdkkivprdc

7F21A1 light chain and heavy chain sequences (CDR sequences shown in bold/underline and VL/VH sequences are shown in capital letters):

7F21A1 Light Chain (SEQ ID NO 15)

msvltqvlglllwltdarcDIQMTQSPASLSVSVGETVTITC**RASEKIYSNLA**WYQQKQGKSPQLLVY **AATNLAD**GVPSRFSGSGSGTQFSLKINNLQSEDFGSYYC**QHFWGTPWT**FGGGTKLEIKRa daaptvsifppsseqltsggasvvcflnnfypkdinvkwkidgserqngvlnswtdqdskdstysmsstltltkdeyerhnsytcea thktstspivksfnrnec

7F21A1 Heavy Chain (SEQ ID NO 16)

mgwswvflfllsvtagvhsQVQLQQSGAELVRPGTSVKVSCRSSGYAFT**NYLIE**WIKQRPGQGLEW IG**VITPGSGGTNYNENFKD**KATLTADKSSNTAYMQLTSLTSDDSAVYFCAR**SFDYDGAWFA Y**WGQGTLVTVSAakttppsvyplapgsaaqtnsmvtlgclvkgyfpepvtvtwnsgslssgvhtfpavlqsdlytlsssvtv psstwpsetvtcnvahpasstkvdkkivprdc

4F3B light chain and heavy chain sequences (CDR sequences shown in bold/underline and VL/VH sequences are shown in capital letters):

4F3B Light Chain (SEQ ID NO 17)

mesqilvlmsllfwvsgtcgDIVMTQSPSSLTVTAGEKVTMSC**KSSQSLLNSGNQKNYLT**WYQQKP GQPPKVLIY**WASTRES**GVPDRFTGSGSGTDFTLTISSVQAEDLAVYYC**QNDYSYPLT**FGTG

TKLELKRadaaptvsifppsseqltsggasvvcflnnfypkdinvkwkidgserqngvlnswtdqdskdstysmsstltltkde
yerhnsytceathktstspivksfnrnec

4F3B Heavy Chain (SEQ ID NO 18)

5 mgwsgififlfsvtagvhsQVQLQQSGAELAKPGASVKMSCKASGFTFT**SYWMH**WVKQRPGQGLE
WIG**YINPSTDYTEYNQKFKD**KATLTADKSSSTAYMQLSSLTSEDSAVYYCAR**RSPFAMDY**W
GQGTSVTVSSakttppsvyplapgsaaqtnsmvtlgclvkgyfpepvtvtwnsgslssgvhtfpavlqsdlytlsssvtvpss
twpsetvtcnvahpasstkvdkkivprdc

10 **3F7A9-1 light chain and heavy chain sequences (CDR sequences shown in bold/underline and VL/VH sequences are shown in capital letters):**

3F7A9-1 Light Chain (SEQ ID NO 19)

mrapaqilgilllwfpgikcDIKMTQSPSSMYASLGERVTITC**KASQDINSFLS**WLQQKPGKSPKTLIY
15 **RANRLVD**GVPSRFSGSGSGQDYSLTISSLEYEDMGIYYC**LQYDDFPYT**FGGGTKLEIKRada
aptvsifppsseqltsggasvvcflnnfypkdinvkwkidgserqngvlnswtdqdskdstysmsstltltkdeyerhnsytceath
ktstspivksfnrnec

3F7A9-1 Heavy Chain (SEQ ID NO 20)

20 mgwsgvflfllsgttgvhsEIQLQQSGPELVKPGASVKVSCKASGYVFT**SYNMY**WVKQSHGKSLEWI
G**YIDPYNGGTRYNQKFKG**KATLTVDKSSSTAYMHLNSLTSEDSAVYYCAR**LDYYGNFY**WG
QGTTLTVSSakttppsvyplapgsaaqtnsmvtlgclvkgyfpepvtvtwnsgslssgvhtfpavlqsdlytlsssvtvpsstw
psetvtcnvahpasstkvdkkivprdc

25 **13F5A5 variable light chain and variable heavy chain sequences (CDR sequences shown in bold/underline and VL/VH sequences are shown in capital letters):**

13F5A5 Variable Light Chain **(SEQ ID NO 21)**

EIVLTQSPGTLSLSPGERATLSC**RASQSVSSSYLA**WYQQKPGQAPRLLIY**GASSRAT**GIPDR
30 FSGSGSGTDFTLTISRLEPEDFAVYYC**QQYGSSPLT**FGGGTKVEIKR

13F5A5 Variable Heavy Chain **(SEQ ID NO 22)**

EVQLVQSGAEVKKPGESLKISCKGSGYSFT**SYWIV**WVRQMPGKGLEWMG**SIYPGDSDARY**
SPSFQGQVTISADKSISTAYLQWSSLKASDTAMYYCAR**QKITMVRGVIITPLDGMDV**WGQG
35 TTVTVSS

13F56 variable light chain and variable heavy chain sequences (CDR sequences shown in bold/underline and VL/VH sequences are shown in capital letters):

13F56 Variable Light Chain **(SEQ ID NO 23)**
EIVLTQSPGTLSLSPGERATLSC**RASQSVSSSYLA**WYQQKPGQAPRLLIY**GASSRAT**GIPDR
FSGSGSGTDFTLTISRLEPEDFAVYYCQQYGSSPTFGPGTKVDIKR

13F56 Variable Heavy Chain **(SEQ ID NO 24)**
QVQLVQSGAEVKKPGSSVKVSCKASGGTFS**SYAIS**WVRQAPGQGLEWMG**KIIPILGITNYA**
QKFQGRVTITADKSTSTAYMELSSLRSEDTAVYYCA**NPLTFDAFDI**WGQGTMVTVSS

16F56 variable light chain and variable heavy chain sequences (CDR sequences shown in bold/underline):

16F56 Variable Light Chain **(SEQ ID NO 25)**
DIQMTQSPSFLSASVGDRVTITC**RASQSISSYLN**WYQQKPGKAPKLLIY**AASSLQS**GVPSRF
SGSGSGTDFTLTISSLQPEDFATYYC**QCGYSTPLT**FGGGTKVEIKR

16F56 Variable Heavy Chain **(SEQ ID NO 26)**
QVQLVQSGAEVKKPGSSVKVSCKASGGTFS**NYAIS**WVRQAPGQGLEWMG**RIIPILGIAHNA**
QKFQDRVTITADKSTSTAYMELSSLRSEDTAVYYCAS**PSSGKGLPYWFFDL**WGRGTLVTVS
S

16F119 variable light chain and variable heavy chain sequences (CDR sequences shown in bold/underline):

16F119 Variable Light Chain **(SEQ ID NO 27)**
EIVLTQSPGTLSLSPGERATLSC**RASQSVSSSYLA**WYQQKPGQAPRLLIY**GASSRAT**GIPDR
FSGSGSGTDFTLTISRLEPEDFAVYYC**QQYGSSLT**FGGGTKVEIKR

16F56 Variable Heavy Chain **(SEQ ID NO 28)**
QVQLVQSGAEVKKPGSSVKVSCKVSGGTFS**NYAIS**WVRQAPGQGLEWMG**RIIPILGIAAYA**
QRFRDRVTITADKFTSTVYMELSSLRSEDTAVYYCAS**GQLGTPYWYFDL**WGRGTLVTVSS

SEQ ID NO 29: 6F3A Variable Light Chain - CDR sequences shown in bold/underline (the hybridomas 6F3A and 6F6B are identical clones):
DIVLTQSPATLSVTPGDSVSLSC**<u>RASQSITNNLH</u>**WYQQKSHESPRLLIK**<u>YASQSIS</u>**GIPSRFR GSGSGTDFTLSINSVETEDFGVYFC**<u>QQSNTWPRT</u>**FGGGTKLEIKR

5

SEQ ID NO 30: 6F3A Variable Heavy Chain - CDR sequences shown in bold/underline (the hybridomas 6F3A and 6F6B are identical clones):
EVQLQQSGAELVKPGASVKLSCTASGFN**<u>IKDTYVH</u>**WVKQRPDQGLEWIG**<u>RIDPAIGNTKYD PKFRG</u>**KATITADSSSNTAYLQLSSLTSEDTAVYYCAR**<u>LSYGNYGLDYSMDY</u>**WGQGTSVTVS
10 S

Example 4

SPR analysis of competition between anti-OSCAR mAb and CoII for binding to OSCAR

The experiment was performed using a Biacore T 100 instrument (GE Healthcare). Human
15 CoII (Sigma, # C5483) was diluted with 10mM NaOAc, pH 4.5 to 10µg/ml and immobilized on a Biacore CM3 sensor chip (GE Healthcare) using amine coupling chemistry (GE Healthcare). Immobilization was performed according to the standard procedure, but at 4ºC in order to prevent aggregation of CoII, with immobilized levels of ca 150 RU on flow cell (FC) 2 and ca 300 RU on FC 4. A blank immobilization was performed on FC 1 & 3, which were
20 used as reference cells. HBS-EP buffer (10mM HEPES, 150mM NaCl, 3mM EDTA, 0.005% Polysorbat P20, pH7.4) was used as running buffer for the immobilization. Buffer containing HBS-EP/+0.1% BSA was used for the competition assay.

The competition experiment was performed at 4 ºC at a flow rate of 50µl/min. The OSCAR-ECD and OSCAR-S1a proteins (SEQ ID NO 2 and SEQ ID NO 3, respectively) were used as
25 analytes at a concentration of 50nM. OSCAR was mixed with various concentrations of anti-OSCAR antibodies ranging from 0 – 5µg/ml prior to injection. Binding curves were measured for 240 s followed by 200 s of dissociation. The chip surface was regenerated for 2 x 20 s with 10mM glycine, pH 2.0 between assays. All assays were performed in duplicates.

30 <u>Results</u>

Human CoII was immobilized to the surface of a Biacore sensor chip by amine coupling. Binding of OSCAR-ECD (SEQ ID NO 2) and OSCAR-S1a (SEQ ID NO 3) and binding of the soluble extracellular domain (ECD) of OSCAR to CoII was studied in the presence or absence of anti-OSCAR antibodies. Two different constructs of OSCAR were analysed:
35 OSCAR-ECD (SEQ ID NO 2) and OSCAR-S1a (SEQ ID NO 3). It was hypothesized that an

antibody inhibiting binding of OSCAR to ColI would lead to a concentration-dependent loss of signal, whereas a non-inhibiting antibody would enhance the signal due to the increased mass of the OSCAR-antibody complex. Of the 5 tested antibodies, 3 fell into one of the two groups. As shown in Table 3, mAbs 13F5A5 and 4F3B inhibited binding of OSCAR to ColI
5 with very little binding left at 5µg/ml antibody. In contrast, 3F7A9 did not interfere with binding since a concentration-dependent increase of the signal was detected.
Further SPR analysis of competition between anti-OSCAR mAbs and ColI/ColII for binding to OSCAR was done as described in Example 13.

10 **Table 3:** SPR analysis of the binding of OSCAR-ECD or OSCAR-S1a to ColI immobilized at two densities

	150 RU colI		300 RU colI	
sample	Binding [RU] no mAb	Binding [RU] 5 µg mAb	Binding [RU] no mAb	Binding [RU] 5 µg mAb
OSCAR 1Sa/13F5A5	11.2	-0.2	22.7	2.0
OSCAR 1Sa/1F5H10-2	11.1	96.6	22.6	158.7
OSCAR 1Sa/2F9G11	11.0	16.5	22.6	28.2
OSCAR 1Sa/3F7A9	10.8	58.9	22.6	92.6
OSCAR 1Sa/4F3B	11.0	-0.3	22.5	1.4
OSCAR ECD/13F5A5	11.0	1.4	24.8	5.1
OSCAR ECD/1F5H10-2	11.0	22.8	25.0	36.7
OSCAR ECD/2F9G11	11.1	133.4	25.0	209.9
OSCAR ECD/3F7A9	11.3	82.5	24.8	130.2
OSCAR ECD/4F3B	10.9	0.9	25.0	3.6

Example 5

Epitope diversity assessed by SPR analysis

15 Antibody binding competition assays were performed by SPR analysis in order to investigate whether the anti-OSCAR mAbs bound simultaneously to OSCAR-ECD (SEQ ID NO 2) or cell-surface OSCAR on human PBMC. An inability of mAbs to bind simultaneously indicates common or overlapping epitopes, though factors such as steric hindrance and conformational changes may contribute.

20

SPR studies were performed using a BiacoreT100 instrument (GE Healthcare). The purified anti-OSCAR mAbs were immobilized on a CM5 sensor chip in individual flow cells. A fixed concentration of OSCAR-ECD in HBS-EP buffer (10mM HEPES, 150mM NaCl, 3mM EDTA and 0.005% Polysorbat P20, pH7.4) was injected prior to injection of a second anti-OSCAR

mAb. All mAbs were tested in all combinations.

Results

Antibodies demonstrating an inhibitory effect on CollI-dependent dendritic cell maturation
5 (described in Example 8) were represented in four bins/competitive groups (Table 4). This data demonstrates that interference with at least four distinct regions in OSCAR will influence the binding and/or activation by CollI. The ligand competing data (described in Example 13) differentiate the mAbs between the four bins.

10 **Table 4:** Binning of mAbs based on simultaneous binding to OSCAR

Bin#1	**Bin# 2**	**Bin# 3**	**Bin# 4**
4F3B	11.1CN5	7F10A1	7F6A1
13F5A5 (Hu)	3F7A9	7F14A1	6F3A
13F56 (Hu)	7F1A1		6F6B
16F119 (Hu)	7F21A		
16F56 (Hu)			

The mAbs in each bin/competitive group were not able to bind simultaneously to OSCAR.

Example 6

Epitope diversity assessed by flow cytometry analysis

15 The aim of the binning study was to group the antibodies into competitive groups/bins according to their ability to inhibit binding of a fluorochrome labelled anti-OSCAR antibody to human monocytes. Inhibition of binding indicates that the two antibodies tested show steric hindrance of simultaneous binding to the target cells and hence belonging to the same bin. An inhibition of binding can be due to binding to the same epitope, binding to overlapping
20 epitopes or binding to two non-overlapping epitopes albeit with steric hindrance of simultaneous binding.
All antibodies shown to inhibit cytokine release by a DC cytokine release assay (described in example 8) were analysed in the binning study.

25 Antibodies

Labelling of mAbs was performed using the Alexa Fluor® 647 Monoclonal Antibody Labeling Kit (Molecular Probes, #A-20186) according to manufactures protocol. The following mouse anti-OSCAR mAbs were labelled: 4F3B, 3F7A9, 6F3A, 6F6B, 7F1A1, 7F6A1, 7F10A1, 7F12A1, 7F13A1, 7F14A1, 7F21A1 and the mAb 11.1CN5. The following human anti-

OSCAR mAbs were labelled: 13F56A6, 13F5A5, 16F56A2 and 16F119A5. The same sets of antibodies were kept unlabelled.

For the quality control and facilitating gating of relevant cell populations, the following mAbs have been used: 7AAD (BD, #51-68981E), anti-CD3-FITC (BD, # 345763), anti-CD19-FITC
5 (BD, # 345776) and anti-CD14-FITC (BD, #345784).

Binding assay

PBMC from healthy individuals were purified as described in Example 1. The cells were re-suspended in washing buffer (1 x PBS (-Ca^{2+}/-Mg^{2+}) + 1% BSA). Viability and cell numbers
10 were calculated using a Cedex cell counter. 10^7 cells (90µl) were treated with 10µl hFcR blocker (Miltenyi, #130-059-901).The cells were incubated 10 minutes at 4ºC, followed by a dilution in washing buffer to 10^6cells/ml. Labelled antibodies were diluted in washing buffer to 2µg/ml and added as 25µl/well in a round bottom 96-well plategiving a final concentration of 0,5ug/ml og labelled antibody. 25µl of unlabelled antibody in 3-fold serial dilutions ranging
15 from 15ug/ml to 250ng/ml (final concentration) were added to the same wells (according to plate outline). Then PBMC were added to all wells at 50µl/well (50.000 cells /well). The cells were incubated in the dark at 4°C for 60 minutes and washed twice in 200µl washing buffer/well. Finally, the cells were washed once in PBS, re-suspended in 100µl PBS/well and fixed with an equal volume of fixation buffer (1 x PBS (-Ca^{2+}/-Mg^{2+}) + 1% Formalin). The cells
20 were kept in the dark at 4ºC until analysis by flow cytometry.

Results and Conclusions

Consistent with the epitope binning results by SPR analysis (described in Example 5), the data from flow cytometry analysis on monocytes ($CD14^+$ cells) showed that the inhibitory
25 mAbs identified in a DC cytokine secretion assay were represented in four bins/competitive groups (Table 5).

The median fluorescence intensity was measured in the presence of various concentrations of unlabelled mAb and used to generate an inhibition curve. The results are shown in Table
30 5. Full inhibition (=at least about 75% inhibition, preferably at least about 90% inhibition, and most preferably at least about 95% inhibition) is shown as grey squares, while partial inhibition (= about 10-50% inhibition, or about 20-40% inhibition), or shown as dotted squares. No inhibition was seen with combinations of the antibodies indicated as white squares. For one of the antibodies, 7F6A1, it was difficult to definitely group it together with
35 the other antibodies tested. Unlabelled 7F6A1 was fully inhibiting binding of labelled 4F3B,

while unlabelled 4F3B only partially inhibited binding of the labelled 7F6A1. Binding of the four labelled human antibodies 13F5A5, 13F56A6, 16F56A2 and 16F119A5 was partially inhibited by F6A1 and 13F5A5 was also able to partially inhibit 7F6A1 which none of the other three human mAbs were able to. Briefly, the mAbs could be grouped into four main groups listed in Table 5. This main grouping is based exclusively on the full inhibition profile. Although it was not possible to label the 6F3A, this mAb has been groped together with 6F6B and 7F6A1. The partial inhibition profile is less clear. Both mAbs in a test were added simultaneously, there should be no preference for one of the antibodies compared to the other. Presumably, labelling of the antibodies with AlexaFlour 647 might have an effect on binding properties. There might be a change in affinity due to labelling of the antibody, or the flexibility of the F(ab) arms may be affected by the labelling.

Table 5: Binning of the mAbs by flow cytometry analysis

Alexa Flour 647 a-OSCAR mAb	Unlabeled a-OSACAR mAb: 3F7A9	4F3B	6F3A	6F6B	7F1A1	7F6A1	7F10A1	7F14A1	7F21A1	13F5A5	13F56A6	16F56A2	16F119A5	11.1CN5
3F7A9-1	■				■				■					■
4F3B		■	▨	▨		■				■	■	■	■	
6F3A	Not tested*:													
6F6B		■	■	■	■	■			■					■
7F1A1	■				■				■					■
7F6A1		▨	■	■		■				▨				
7F10A1							■	■						
7F14A1							■	■					▨	▨
7F21A1	■		■	■	■				■					■
13F5A5		■				▨				■	■	■	■	
13F56A6		■				▨				■	■	■	■	
16F56A2		■				▨				■	■	■	■	
16F119A5		■				▨				■	■	■	■	
11.1CN5	■				■			▨	■					■

Not tested*: The antibody was labelled twice with no success

■ full mutual inhibition ▨ partial inhibition

BINNING-GROUPS	Group 1:	Group 2:	Group 3:	Group 4:
	4F3B	3F7A9-1	7F10A1	7F6A1
	13F5A5	7F1A1	7F14A1	6F3A
	13F56A6	7F21A1		6F6B
	16F56A2	11.1CN5		
	16F119A6			

Black squares indicate full inhibition and the dashed squares indicate partial inhibition.

Example 7

Epitope mapping by peptide array

A preliminary epitope prediction of the anti-OSCAR mAb 4F3B was done using a peptide walking technique. A peptide array was synthesised based on SEQ ID NO 2 with C to A mutations for all cysteins. A 20- (20mer), 16- and 12 amino acid residue peptide walk was used in the design of the peptide array. The frame walking was set to 2 residues. The array was screened using the fluorescein-labelled mAb 4F3B.

Synthesis of epitope arrays

The epitope mapping arrays were synthesized on cellulose sheets (Aims-Scientific) using an array synthesizer (Multipep Spot, Intavis) according to the manufacturers protocol. Fmoc-amino acids (Novabiochem) were dissolved in N-methylpyrrolidinone (NMP) containing 0.3M hydrobenzotriazole (HOBt) to a final concentration of 0.3M. Coupling was done by activating with diisopropylcarbodiimide (DIC). De-protection of the Fmoc group was done by 20% piperidine in NMP. The individual sequences were designed using the array synthesiser software. After synthesis, the protecting groups were removed by treatment of the sheets with 95% trifluoroacetic acid (TFA) containing triisopropylsilane (TIPS) for 60 min. The sheets were washed with dichloromethane (DCM) and N-methylpyrrolidinone (NMP) and finally with water.

Labelling of antibody

The stock of the 4F3B mAb was subjected to gel-filteration chromatography, using a NAP5 column (GE Healthcare) to replace the buffer with 1% $NaHCO_3$ according to the manufacturer recommendations. This was followed by adding 25 mole equivalents of 5(6)-carboxyfluorescein N-hydroxysuccinimide ester (Sigma, #C1609) dissolved in DMSO. The coupling reaction continued for 2 hours followed by gel-filtration against the washing buffer (50mM TRIS, pH7.4, 0.15M NaCl, 0.1M ArgHCl, 0.05% Tween 20) in order to removed uncoupled fluorescein.

Screening of arrays

Screening was performed with 10µl of fluorescein-labelled 4F3B in 30 ml incubation buffer (0.5% BSA, 50mM TRIS, pH7.4, 0.15M NaCl, 0.1M ArgHCl, 0.05% Tween 20). The sheets were incubated for 1-2 hours, followed by washing five times with the washing buffer. The sheets were scanned using a laser scanner (Typhoon 9410, GE Healthcare) and the image file (.gel format) was analysed using an ArrayPro Analyzer (Media Cypernetics). The

fluorescence intensity was measured and transformed into digits which were exported to GraphPad Prism 5 for further analysis.

Results

5 The array analysis revealed that the mAb 4F3B binds to the regions corresponding to amino acid residues 29-48 and 129-146.

The structure of OSCAR has not yet been solved. Using an available 3D structure of KIR2DL2 as a template, a homology model of OSCAR-ECD was built. According to the model, the binding regions for mAb 4F3B are located in each of the Ig-like domains.

10 The technique used here allows a preliminary prediction of linear epitopes. For accurate eptope mapping techniques such as hydrogen exchange and mass spectrometry (HX-MS) and/or X-ray crystallography of complexes between OSCAR and specific mAbs or fractions thereof (e.g. Fab fragments) can be employed.

An OSCAR variant having a Cys-to-Ala mutation in position 83 has been produced. This 15 C83A OSCAR variant can potentially be useful for protein structure determination (e.g. X-ray analysis), protein-ligand interface determination and epitope mapping.

Example 8

Inhibition of ColI-induced DC maturation and cytokine release by anti-OSCAR mAbs

20 Brand et al (Eur. J. Immunol., 1998, 28: 1673-1680) have shown that Collagen I induces maturation of monocyte-derived DC (moDC). These cells exhibited allostimulatory capacity. However, DC receptors mediating ColI-induced effects were not identified. Merck et al (Blood, 2005, 105: 3623-3632) have shown that cross-linking of OSCAR with specific antibodies can induce maturation of moDC. As ColI was identified as an interaction partner 25 for OSCAR, we hypothesised that ColI-induced maturation of moDC is mediated by OSCAR. We have developed an assay allowing to test this hypothesis. It was found that immature moDC plated on un-treated (i.e. having neutral surface charges) virgin polysterene multi-well plates (e.g. from IWAKI) were best suited for coating with ColI providing maturation of moDC. Blocking of OSCAR with polyclonal antibodies abolished maturation of moDC plated on ColI. 30 We therefore concluded that the assay was suitable as a functional screen for neutralizing anti-OSCAR mAbs.

Methods

Human PBMC were isolated as described in Example 1. Monocytes were purified from 35 PBMC using MACS $CD14^{+}$ beads (Miltenyi MicroBeads, #130-050-201). The monocytes

were seeded at a cell density of $2X10^6$ cells/ml and cultured for 3 days in the presence of 12.5 ng/ml IL-4 (R&D Systems, #204-IL-010) and 25 ng/ml GM-CSF (eBioscience, #14-8339) at $2x10^6$ cells/ml for 3 days. The monocytes differentiated into immature DC, which were non-adherent cells of irregular shape that expressed high levels of DC-SIGN. The immature
5 DC were pre-incubated for 1h with anti-OSCAR or control antibodies and subsequently seeded into plates (IWAKI, #1820-024) coated with ColI (Sigma, #C5483) or FCS (control) and incubated overnight. The maturation state of the DC (i.e. expression of HLA-DR, CD83, CD86 and DC-SIGN) was assessed by flow cytometry. Secretion of cytokines was analysed by ELISA (R&D systems). TNF-α and IL8 were identified as the cytokines providing the best
10 window (ColI versus FCS treated DCs) and thus were chosen as readouts in the screen. All mAbs and ColI used in the assay were endotoxin-free. DC plated onto FCS-coated plates were used to control for agonistic properties of mAbs.

Results

15 All anti-OSCAR mAbs which showed binding human monocytes were tested for inhibitory properties in this assay at multiple doses. IL8 and TNF-α concentrations were plotted as a function of log [conc. of mAb in nM]. The curves were fitted using the "log[inhibitor] vs. response- variable slope" fit (GraphPad Prism 5 software). IC_{50} values were defined as the concentration of mAb inhibiting IL8 release to 50% of the level induced by ColI. Isotype
20 control IgGs had no inhibitory activity. Several anti-OSCAR mAbs with comparable affinities did not inhibit cytokine release. All mAbs were screened using moDC from several donors. Table 6 summarises the IC_{50} values which vary between donors.

Table 6: IC$_{50}$ values (IL-8) for different anti-OSCAR mAbs

IC50 (nM)	Donor 1	Donor 2	Donor 3	Donor 4	Donor 5	Donor 6
Bin 1						
4F3B	0.19	n.d.	0.40	0.12	0.43	0.24
16F56A2 (Hu)	n.t.	n.t.	n.t.	0.06	0.10	0.21
16F119A5 (Hu)	n.t.	n.t.	n.t.	0.02	0.46	1.12
13F5A5 (Hu)	2.0	n.d.	n.d.	<0.01	0.23	0.06
13F56A6 (Hu)	n.t.	n.t.	1.4	1.54	2.0	1.88
Bin 2						
7F1A1	<0.1	<0.1	0.1	0.03	n.t.	n.t.
7F21A1	n.d.	0.02	0.64	0.11	n.t.	n.t.
3F7A9	0.16	0.06	0.41	0.06	0.05	0.10
11.1CN5	n.t.	n.t.	n.t.	0.31	>20	0.84
Bin 3						
7F14A4	0.23	n.d.	0.62	n.t.	n.t.	n.t.
7F10A1	0.49	n.d.	1.59	2.06	n.t.	n.t.
Bin 4						
7F6A1	n.d.	n.d.	0.91	n.t.	2.49	1.28
6F6B	n.t.	n.t.	n.t.	0.86	>20	6.56
6F3A	n.t.	n.t.	n.t.	0.69	>20	8.06

n.d. not determined.
5 n.t. not tested.

Conclusions

Nine mouse- and four human inhibitory mAbs were identified representing 33% and 36%, respectively, of mAbs which demonstrated strong binding to monocytes. The inhibitory anti-
10 OSCAR mAbs had no agonistic properties. The comparative potency of the mAbs is the same even though the IC$_{50}$ values vary between donors.

Example 9

Anti-OSCAR mAb inhibit DC maturation and hence their ability to promote T-cell
15 **proliferation in an allogenic mixed lymphocyte reaction.**

The ability of the coll- matured DC to promote T-cell proliferation has been assessed using an allogenic mixed lymphocyte reaction (MLR).

20 Method of allogenic Mixed Lymphocyte Reaction (MLR)

(1) Purification of CD14+ cells, differentiation and maturation of DC

CD14+ monocytes were purified from buffy coats of healthy donors. The cells were enriched using RosetteSep Human Monocyte Enrichment Cocktail (StemCell Technology, #15068)

according to the manufacturers protocol. Residual erythrocytes were lysed using RBC lysis buffer (eBioscience, # 00-4333-57) for 10 min at room temperature. Monocytes were washed twice with the buffer containing PBS, 2% FBS and 10mM EDTA, collected by centrifugation and re-suspended at the concentration of $6.7x10^5$ cells/ml in the DC differentiation medium
5 containing RPMI1640 with 2mM L-glutamine (Glutamax™, Gibco, # 61870), 10% FBS, 50ng/ml human recombinant GM-CSF (R&D, #215-GM) and 50ng/ml human recombinant IL-4 (PeproTech, #200-04). $1,2x10^7$ cells were seeded onto a 92x17 mm tissue culture plate (Nunc, # 150350) and cultured for five days in 5% CO_2 at 37°C. The medium was renewed after two days of culturing.
10

After five days, non-adherent immature DC (iDC) were collected, extensively washed in a growth medium without GM-CSF and IL-4 (RPMI1640 with 2mM L-glutamine, 10% FBS), and re-suspended at $4x10^5$/ml in the same growth medium. An aliquot of cells was taken for analysis of the surface markers expression using flow cytometry (see FACS analysis). The
15 cells were pre-incubated for 30 min with anti-OSCAR mAbs prior to transfer to 24 well microplates (IWAKI, #1820-024), which were coated for 3 hours at room temperature with 300µl/well of 10µg/ml human CollI (Sigma, #C5483) and rinsed thoroughly with PBS, or to the uncoated wells. Unless indicated, $4x10^5$ cells per well were used. The DC growth/survival factors (50ng/ml GM-CSF and 50ng/ml IL-4) or inducers of maturation (5µg/ml LPS, Sigma,
20 #SC-3535) were added to the control wells. The cells were cultured for two days in 5% CO_2 humidified cell incubator at 37°C. Non-adherent cells were collected, extensively washed and resuspended in RPMI1640 supplemented with 10% FBS and 2mM L-glutamine (further referred as "T-cell growth medium") for further use in MLR. Expression of cell surface markers was analysed by flow cytometry (see FACS analysis). Detection of cytokines in the
25 conditioned medium was performed using Bio-Plex multiplex analysis (see Analysis of Cytokine secretion).

(2) Mixed lymphocyte reaction (MLR)

$CD4^+$ human T-cells were isolated from buffy coats of healthy donors using RosetteSep
30 Human $CD4^+$ T cell Enrichment Cocktail (StemCell Technologies, #15062) according to the manufacturers protocol followed by Histopaque density gradient purification (Sigma, #10771). The $CD4^+$ cells were recovered from the interphase, thoroughly washed with PBS/2% FCS and re-suspended in T-cell growth medium. The purity of the T-cells was analysed by flow cytometry (see FACS analysis).
35 To set up MLR, DC were mixed with allogenic $CD4^+$ T-cells in a ratio 1:100 (10^3 DC:10^5 T-

cells per well of a 96-well plate). The cells were cultured in 5% CO_2 humidified cell incubator at 37°C for 5 days. T-cells cultured without DC served as negative control. At day 4 conditioned medium was collected for analysis of cytokine secretion (see Analysis of cytokine secretion). The cells were then pulse-labelled with 0.5µCi/well ^{3}H-Thymidine (Perkin Elmer, 14.4 Ci/mmol) for 18 hours. The cells were collected using a Tomtec cell harvester and lysed in distilled water. Incorporated thymidine was captured on glass fibre filters. The filters were air-dried for 20 hours at room temperature prior to quantification using a TopCounter scintillation counter (Perkin Elmer Topcount NXT).

(3) FACS analysis

The following antibodies were used for phenotype analysis of moDC:
Cocktail #1: CD14-APC (BD, #555399)/HLA-DR-FITC (BD, # 335831)/ Live/Dead Cell Staining DCS-Near-IR (Molecular Probes, # L10119)/OSCAR-PE (Beckton Coulter, clone 11.1CN5).
Cocktail #2: CD86-PE /CD83-APC /209-PerCP-Cy5.5 (BD, #84366)
To estimate the purity of the isolated CD4$^+$ T-cell population the following antibodies were used:
Cocktail #1: CD4-FITC (BD, #555348)/CD8-PE (BD, #555635)/CD19-PE-Cy7 (BD, #557835) /CD3-APC (BD, # 555335)/CD14-APC-H7 (BD, #641394).
Cocktail #2: CD4-APC (BD, #555349)/Live/Dead Cell Staining DCS-Near-IR (Molecular Probes cat# L10119).
The cells were analysed using an LSRII flow cytometer. The data were treated using FACS Diva and FlowJo softwares.

(4) Analysis of Cytokine secretion

The cytokine secretion was assessed using a Bio-Plex system for multiple cytokine analysis (BioRad). 50µl of cell culture supernatants were analysed. Secreton of IL23(p19/p40) was analysed by ELISA (eBiosciences, #88-7239) with the capture anti-IL12p40 and detection anti-p19 Ab according to the manufacturers protocol. Data analysis was performed using the GraphPad Prism 5.0 software.

Results

1. Col1 induced the maturation of DCs and cytokine release, which can be inhibited by anti-OSCAR mAbs (Table 7).

Non-adherent immature DC (iDC) differentiated from monocytes in the presence of GM-

CSF/IL4 were transferred either onto the plates coated with ColI and maintained in the full growth medium, or onto uncoated plates in the presence of LPS (positive control, "classical" mature DC) or uncoated plates in the presence of GM-CSF/IL4 (negative control, to maintain the iDC phenotype). The expression of the maturation cell surface markers was analysed by FACS. Cells propagated on ColI were phenotypically similar to the classical LPS-matured DC ($CD80^{high}/CD83^{high}/CD86^{high}$/ $DC\text{-}SIGN^{+}/CD14^{-}$), while the negative control cells remained $CD80^{low}/CD83^{low}/CD86^{low}$/ $DC\text{-}SIGN^{+}/CD14^{-}$. The cytokine secretion profile of the ColI-matured DC was somewhat different from that of the LPS-matured DC. In contrast to the LPS-matured DC, the ColI-matured DC expressed IL23, but not IL12 and higher amounts of IL2 as compared to the LPS-matured DC. Multiple pro-inflammatory cytokines and chemokines were detected in supernatants from both, LPS- and ColI-matured DC. The ColI-induced cytokine release was inhibited by anti-OSCAR mAbs 4F3B and 3F7A9. The non-inhibitory anti-OSCAR mAb 9F11A2 served as an isotype control (Table 7).

2. ColI-matured DC stimulated T-cell proliferation and cytokine secretion in an allogenic MLR assay, which can be inhibited by anti-OSCAR mAbs (Table 8 and 9) .
To address functionality of the ColI-matured DC, an allogenic mixed lymphocyte reaction has been set. ColI-matured DC stimulated T-cell proliferation at least as efficient as the classical LPS-matured DC. The proliferation of T-cells was significantly lower when the cells were co-cultured with DC treated with the anti-OSCAR mAbs 4F3B and 3F7A9 (Table 8). The cytokine secretion profile of the T-cells is shown in Table 9. Even though the DC used in MLR were not PFA-fixed, the T-cell growth medium does not support survival of DC, thus the contribution of the DC-derived cytokines is minimal (if any).

Conclusion

These data suggest that anti-OSCAR mAbs inhibit DC maturation, and hence suppress their ability to promote an allogenic T-cell proliferation.

Table 7: Secretion of cytokines by DC

	iDC	LPS-DC	Coll-DC	Coll-DC 0.5µg/ml 4F3B	Coll-DC 5 µg/ml 4F3B	Coll-DC 0.5 µg/ml 3F7A9	Coll-DC 5 µg/ml 3F7A9	Coll-DC 0.5µg/ml 9F11A2	Coll-DC 5 µg/ml 9F11A2
IL-2	33±8	26±5	28±1	17±1	28±1	22±1	19	26±4	33±3
IL-3	315±22	571±26	380±4	569±15	786	517	525±55	429±7	460±80
IL-4	2	2	2	2	3	2	2	2	2
IL-5	19±17	170±13	22±3	3	2	2	3±1	22	21±3
IL-6	2428±2	22547±364	8565±176	1729±98	543±92	3176±282	3658±255	6750±501	6589±316
IL-8	11406±486	20286±508	20167±523	11809±458	4279±114	16274±163	15378±193	19911±700	19751±587
IL-10	239±48	12049±126	2801±186	86±11	79±2	208±26	176±12	2330±48	2231±132
IL-12p40	536±35	39407±17771	46361±406	596±49	531±61	13398±1279	3399±233	46937±2298	44221±2396
IL-12p70	72±34	14287±316	100±6	40±2	43±6	50±3	43	87	95
IL-23*	ND	696±9	272±6	ND	ND	10±5	ND	289±40	228±17
IL-13	21±11	1658±7	1034±34	11	9	44±6	19±2	1039±35	956±68
IL17	50±6	51±1	46±8	50	62±5	53±2	56±6	44±5	52±4
INF□	78±37	4937±870	69	69	87	78±37	95±12	95±12	78±12
MCP-1	1968±121	1790±83	2606±51	207±10	89±12	525±37	453±19	2561±88	2405±212
RANTES	64±15	4479±193	2201±132	55	34±3	62±2	46±1	2131±53	1872±32
TNF□	367±101	44763±1881	31108±1967	78	61	217±12	104±12	28719±2669	26543±1310

Data represent mean ± SD (n=3), pg/ml; *IL-23 was measured using p40/p19 specific ELISA; ND: not detectable

Table 8: Allogenic mixed lymphocyte reaction: proliferation of T-cells stimulated by DC which were matured in the presence of the indicated agents

	CPM, mean ± SD (n=3)
Immature DC	27032 ± 5148
LPS matured DC	31155 ± 2768
Coll matured DC	38532 ± 2445
Coll matured DC + 0.5 µg/ml 4F3B	9498 ± 1546
Coll matured DC + 5 µg/ml 4F3B	7404 ± 1225
Coll matured DC + 0.5 µg/ml 3F7A9	18437 ± 1451
Coll matured DC + 5 µg/ml 3F7A9	15201 ± 3183
Coll matured DC + 0.5 µg/ml 9F11A2	37780 ± 3396
Coll matured DC + 5 µg/ml 9F11A2	40124 ± 3930
No DC	231 ± 31

Table 9: Secretion of cytokines by T-cells in allogenic mixed lymphocyte reaction

	Basal level	iDC-induced	LPS-DC induced	Coll-DC induced	Coll-DC 0.5µg/ml 4F3B	Coll-DC 5 µg/ml 4F3B	Coll-DC 0.5 µg/ml 3F7A9	Coll-DC 5 µg/ml 3F7A9	Coll-DC 0.5µg/ml 9F11A2	Coll-DC 5 µg/ml 9F11A2
IL-2	23±5	115±30	229±27	432±101	118±39	89±11	165±54	100±33	393±31	419±153
IL-3	675±52	759±27	701±76	570±305	725±58	739±73	593±236	737±62	698±16	507±188
IL-4	2	4±1	3	3	2	3	3	2	3	3
IL-5	2	47±14	419±139	470±146	70±38	34±16	205±75	106±15	385±148	446±53
IL-6	28±6	676±38	1504±256	687±206	162±58	196±41	140±69	126±12	986±255	806±273
IL-8	209±82	15747±451	3854±69	7452±1017	8907±594	7801±2189	8508±2079	6666±749	8907±594	7801±2189
IL-10	22±2	476±83	469±124	808±551	56±7	55±19	378±96	132±59	869±171	990±253
IL-12p40	290±17	624±104	964±57	772±260	383±9	370±18	388±151	410±41	1025±244	725±194
IL-12p70	34±2	44±15	47±7	52±6	34±3	35±3	40±5	38±3	98±34	116±74
IL-13	4±1	441±8	1310±246	1755±90	285±136	163±76	650±288	213±21	1840±310	1924±179
IL17	44±8	59±4	129±40	76±23	55±6	59±3	65±4	63±12	104±13	92±9
INFg	41±7	202±75	718±374	1113±317	82±5	71±7	84±40	82±46	3667±1331	1569±882
MCP-1	8±3	428±53	23±1	31±5	550±317	859±224	65±24	148±62	79±10	44±20
RANTES	79±39	531±87	803±52	1281±523	167±56	181±62	340±60	168±56	1448±397	1289±47
TNFa	54±4	561±63	356±104	495±91	298±24	281±39	228±98	228±34	713±68	675±278

Data represent mean ± SD (n=3), pg/ml

Example 10

Anti-OSCAR mAbs reduce viability of DC propagated on Col

ColI supported survival of iDC at the conditions of growth factor withdrawal. It has been investigated whether the effect is mediated by OSCAR.

Methods

iDC were differentiated from $CD14^+$ monocytes as described in Example 9. After five days, non-adherent iDC were collected and extesively washed to remove traces of GM-CSF and IL4. The cells were re-suspended at $2.5x10^6$ cells/ml in the growth medium containing RPMI 1640 with Glutamax™, (Gibco, #61870) supplemented with 10% FBS. Prior to seeding, aliquots of $5x10^5$/200µl cells were pre-incubated for 30min at 37°C with 300µl of anti-OSCAR mAbs (diluted with growth medium) or with 300µl of growth medium without mAb as untreated control. After incubation the cells were transferred to 24 well microplates (IWAKI, #1820-024), which were coated for 3 hours at room temperature with either 300µl/well of 10µg/ml human ColI (Sigma, #C5483) or 20µg/ml fibronectin (Sigma, #F0895) and rinsed thoroughly with PBS, or to the uncoated wells. 500µl of the growth medium was added per well. As positive control, 50ng/ml GM-CSF was added to the indicated wells. The cells were propagated for three days in a 5% CO_2 humidified cell incubator at 37°C.
Metabolic activity was analysed as a surrogate marker of cell viability using Alamar Blue dye (Invitrogen, # Dal1025) according to the manufacturers protocol. Fluorescence was measured at excitation 544nm/emission 590nm using a NovoStar fluorescence plate reader (BMG). Data analysis was performed using the GraphPad Prism 5.0 software.

Results

The effect of ColI-OSCAR interaction on the viability of DC under conditions of growth factor withdrawal has been addressed. Metabolic activity of the cells was used a surrogate marker of cell viability. Monocyte-derived iDC cultured on ColI were at least as viable as the cells cultured in the presence of the growth factor GM-CSF. In contrast, the cells plated onto another matrix protein, fibronectin, or onto un-coated plastic demonstrated poor viability. Anti-OSCAR mAbs suppressed metabolic activity of the DC plated on ColI indicating that the survival-promoting effect of ColI is mediated by OSCAR (Table 10).

Conclusion

ColI-OSCAR interaction promotes the survival of DCs under the conditions of grow factor withdrawal and the effect can be suppressed by anti-OSCAR mAbs.

Table 10: ColI-OSCAR interaction supports survival of DC under conditions of growth factor withdrawal

	RFU (Alamar Blue, 544/590 nm) Mean ± SD (n=3)
Uncoated	1763 ± 111
Uncoated; +GM-CSF	2629 ± 112
Fibronectin	1644 ± 529
ColI	3407 ± 173
ColI + 0.1 µg/ml 3F7A9	1115 ± 61
ColI + 1 µg/ml 3F7A9	1084 ± 76
ColI + 0.1 µg/ml 4F3B	1344 ± 78
ColI + 1 µg/ml 4F3B	1061 ± 60
ColI + 0.1 µg/ml IgG_1	3072 ± 389
ColI + 1 µg/ml IgG_1	3046 ± 219

5 **Example 11**

Anti-OSCAR mAbs inhibit ColI/Col II-induced cytokine release from RA synovial fluid cells

It has been shown that *in vitro* ColI stimulate secretion of pro-inflammatory cytokines by SFMC of RA patients (Jeng KC et a.l (1995) Immunol. Lett. 45 (1-2): 13-17). Since in RA
10 joints ColI and ColII can be exposed due to progressive tissue damage, contact with immune cells and their activation by these matrix proteins may have physiological implications. Here, we have tested whether activation of of RA synovial fluid cells by ColI /ColII is, at least in part, mediated by OSCAR and can be inhibited by anti-OSCAR mAbs.

15 Preparation of RA synovial fluid cells and isolation of RA SFMCs

Synovial fluid from knee joints of RA patients was collected by needle aspiration at Peking University People's Hospital Hospital (Beijing, China). The RA synovial fluid cells were collected by centrifugation, re-suspended in RPMI1640 medium and purified by Ficoll density gradient centrifugation. RA synovial fluid mononuclear cells (RA SFMCs) were recovered
20 from the interphase, washed twice with PBS and re-suspended in RPMI1640 supplemented with 10% FBS and 2mM L-glutamine.

Analysis of CoII-induced cytokine secretion

OSCAR ligand CoII (Millipore, #cc050), CoIII (Millipore, #cc052) , or the control protein collagen V (Millipore, #cc077), were immobilized on a 96-well plate (Costar, #3361) at 100 µl/well of 10µg/ml protein solution in PBS for 4h at 37◦C. RA synovial fluid cells ($3x10^5$/200µl
5 per well) or RA SFMCs ($1x10^5$/200µl per well) were pre-incubated with the anti-OSCAR mAbs or isotype control mAbs for 1h prior to be transferred onto the collagen-coated plate. After 24 hours incubation, the cell culture supernatants were analysed by ELISA for the presence of TNF-α, IL-6 and IL-8.

10 **Results**

Plate-bound CoII/CoIII induced robust release of TNFα, IL-6 and IL-8 from RA synovial fluid cells as well as from the isolated RA SFMCs, which can be inhibited by anti-OSCAR mAbs. For example, two mouse anti-OSCAR mAbs 3F7A9-1 and 4F3B, but not the isotype control anti-OSCAR mAb 9F11A2 (mIgG1), inhibited CoII-induced TNFα and IL-8 secretion from RA
15 SFMCs. The inhibition was not dose-dependent within the tested range of mAb concentrations presumably due to a high potency of these mAbs: the maximal effect was reached already at 0.1µg/ml. (Table 11 and 12).
Furthermore, by using RA synovial fluid cell, 4F3B (murine mAb) and 13F5A5 (Human mAb), but not their corresponding isotype control, inhibited CoII/CoIII-induced inflammatory cytokine
20 release (Table 13). These data suggest that blocking of the interaction between OSCAR and CoII/CoIII can prevent activation of the myeloid cells present in the RA synovial fluid.

Conclusion

Anti-OSCAR mAbs inhibit CoII/CoIII-induced inflammatory cytokine release from RA synovial
25 fluid cells, suggesting blocking OSCAR can inhibit the induction of inflammatory responses in myeloid cells present in RA synovial fluids.

Table 11: Anti-OSCAR mAbs inhibit CoII-induced IL-8 secretion from RA SFMCs

anti-OSCAR mAbs	% of inhibition		
	0.1 µg/ml	1 µg/ml	10 µg/ml
3F7A9-1	60	60	63
4F3B	64	81	88
9F11A2	24	10	4

RA SFMCs: RA synovial fluid mononuclear cells

30

Table 12: Anti-OSCAR mAbs inhibit ColI-induced TNFα secretion from RA SFMCs

anti-OSCAR mAbs	% of inhibition		
	0.1μg/ml	1 μg/ml	10μg/ml
3F7A9-1	91	93	91
4F3B	95	100	100
9F11A2	25	13	0

RA SFMCs: RA synovial fluid mononuclear cells

Table 13: Anti-OSCAR mAbs inhibit ColI/ColII-induced TNFα secretion from RA synovial
5 fluid cells

anti-OSCAR mAbs (10 μg/ml)	% of inhibition	
	ColI-induced TNFα	ColII-induced TNFα
mIgG1	26	15
4F3B	90	95
9F11A2-hIG1.1	20	20
13F5A5-hIgG1.1	100	85

Example 12

Osteoclast formation assay

Osteoclast formation assays can be used to detect if an OSCAR mAb has the ability to
10 reduce bone erosion. The mAbs with the ability to inhibit bone erosion by at least 10%, preferably at least 20%, preferably at least 25%, preferably at least 30%, preferably at least 40%, preferably at least 50%, preferably at least 75%, and most preferably at least 90%. Osteoclasts are multinucleated cells derived from myeloid progenitors that are capable of eroding bone. A standard protocol for generating osteoclasts *in vitro* is to culture monocytes
15 for 7-14 days in the presence of exogenous recombinant M-CSF and soluble RANKL, both factors are required and sufficient for OC formation. During such culture conditions monocytes typically change characteristics as follows:

Day 0-6: low levels of cell fusion and TRAP activity

Day 6-8: preosteoclast characteristics with evident cell fusion, increased TRAP
20 activity, and cathepsin K expression

Day 9- : mature osteoclast characteristics, multinucleation, bone resorption, high TRAP activity, and high cathepsin K expression.

In this example, we tested the effect of anti-OSCAR human mAbs in such a standard osteoclast formation assay using PB CD14+ monocytes from peripheral blood of healthy donors.

5 Methods

(1) Purification of monocytes

Primary human monocytes are purified by a positive selection method using anti-CD14 mAb coated microbeads. Briefly, PBMCs are isolated from buffy coats using Ficoll density gradient centrifugation. Thereafter, monocytes are isolated using CD14 microbeads followed by 10 MACS column separation (Miltenyi Biotec, #130-050-201) according to the manufacturers recommendations.

The OC formation assay is set up in 96 well plate formats.

(2) OC formation assay

15 The OC formation assay is set up in a 96 well plate format. A starting concentration of 60 000 monocytes/well is used. The cells are cultured either on uncoated plastic in a final volume of 200µl of the growth medium alone (IMDM, 2% human serum, 10% FCS, P/S, 2mM L-glutamine), or in the growth medium supplemented with recombinant human M-CSF (25ng/ml) and soluble RANKL (100ng/ml).

20 To evaluate whether anti-OSCAR mAbs impact osteoclast formation and/or active bone resorption, each mAb is added at 10µg/ml at the initiation of culture. The mAbs with hIgG1.1 backbone used in this assay are non-depleting antibodies.

At the end of the assay (day 9-12), supernatants are harvested and the adherent cells grown on plastic and bone slices are washed 3 times with 2 ml PBS and fixed with 4% 25 paraformaldehyde (PFA) in PBS for 5 min. After washing in ddH_2O the cells are stained for TRAP using a leukocyte acid phosphatase kit and counter-stained with hematoxyline according to instructions provided by the manufacturer (Sigma-Aldrich).

Results

30 After 9 days culture, normal $CD14^+$ monocytes were able to differentiate into robust mature osteoclasts in the presence of both M-CSF and RANKL. Cells treated with 13F5A5-hIgG1.1 and Cycrosporine A (CsA) showed inhibitory effect on osteoclastogenesis, whereas 11.1CN5 (Beckman Coulter, #PN A24987), 9F11A-hIgG1.1 (non-blocking mAb) and isotype control mAb ATNP-hIgG1.1 showed no impact on osteoclast formation. Osteoclast formation/activity 35 was measured by TRAP (Osteocalst marker) staining (Figure 1) and soluble TRAP5B

secretion (Table 14).

Conclusion

Anti-OSCAR mAb (i.e.13F5A5-hIgG1.1) inhibited Osteoclast formation from normal
5 monocytes, suggesting blocking of OSCAR will reduce osteoclastogenesis and bone erosion in RA.

Table 14. Anti-OSCAR mAb 13F5A5 inhibited TRAP5b production from osteoclasts formed from normal monocytes

Tested mAbs	% inhibition on TRAP5b
ATNP-hIgG1.1	5
13F5A5-hIgG1.1	40
mIgG1	10
11.1CN5	0
CsA	45

10

Example 13

Competitive binding- SPR analysis of competition between anti-OSCAR mAb and CollI for binding to OSCAR

The experiment was performed using a Biacore T 100 instrument (GE Healthcare). Human
15 collagen 1 (Sigma, # C5483) and human collagen 2 (Millipore #CC052) were diluted with 10mM NaOAc, pH 4.5 and immobilized on a Biacore CM3 sensor chip (GE Healthcare) using amine coupling chemistry (GE Healthcare). Immobilization was performed according to the standard procedure, but at 5ºC in order to prevent aggregation collagens, with immobilized levels of ca 250 RU on flow cells (FC) 2 and 4. A blank immobilization was performed on FC
20 1 & 3, which were used as reference cells. HBS-EP buffer (10mM HEPES, 150mM NaCl, 3mM EDTA, 0.05% Polysorbat P20, pH7.4) was used as running buffer for the immobilization. Buffer containing HBS-EP/+0.1% BSA was used for the competition assay. The competition experiment was performed at 5 ºC at a flow rate of 50µl/min. OSCAR-ECD (SEQ ID NO 2) was used as analytes at a concentration of 50nM. OSCAR was mixed with
25 various concentrations of anti-OSCAR antibodies ranging from 0 – 10µg/ml prior to injection. Binding curves were measured for 360 s followed by 240 s of dissociation. The chip surface was regenerated for 2 x 20 s with 10mM glycine, pH 2.0 between assays.

Results

Human collagen I and II were immobilized to the surface of a Biacore sensor chip by amine coupling. Binding of OSCAR-ECD (SEQ ID NO 2) to collagens 1 & 2 was studied in the presence or absence of anti-OSCAR antibodies. It was hypothesized that an antibody
5 inhibiting binding of OSCAR to collagen would lead to a concentration-dependent loss of signal, whereas a non-inhibiting antibody would enhance the signal due to the increased mass of the OSCAR-antibody complex. Of the 15 tested antibodies, 13 fell into one of the two groups. As shown in Table 15, mAbs 16F119, 16F56, 13F56A6, 13F5A5, 16F18, 4F3B and 6F6B inhibited binding of OSCAR to collagen with very little binding left in the presence
10 of 10µg/ml antibody. In contrast, 7F10A1, 7F14A1, 3F7A9, 7F21A1, 7F1A1 and the prior art antibody 11.1CN5 did not interfere with binding since a concentration-dependent increase of the signal was detected.

Conclusions

15 Anti-OSCAR mAbs in epitope Bin#1 and #4, but not Bin #2 (incl. 11.1CN5) or #3 compete the binding of Collagens to OSCAR. The ligand competing data functionally differentiate the mAbs between the four epitope Bins.

Table 15. The effect of anti-OSCAR mAbs from the four different epitope binds on the
20 binding of OSCAR-ECD to CollI/CollII by SPR analysis.

		collagen I		Collagen II	
Epitope bin#	**mAb**	**Binding w/o mAb**	**binding with 10 µg/mL mAb**	**Binding w/o mAb**	**binding with 10 µg/mL mAb**
1	4F3B	10.9	0.7	17.8	6.7
1	13F5A5	9.4	-1.4	14.3	1.3
1	13F56A6	9.3	-2.1	13.7	-1.4
1	16F18	8.8	-0.3	12.2	0.1
1	16F56	8.4	-3.2	10.9	-1.9
1	16F119	8.4	-3.4	10.6	-1.5
2	3F7A9	10.1	132.2	17.6	246.4
2	7F1A1	10.8	144.6	17.6	335.4
2	7F21A1	11	143.5	18.2	319.1
2	11.1CN5	10.9	145.9	18.3	304
3	7F10A1	10.5	55	17.6	102.4
3	7F14A1	10.7	56.3	18.1	83.6
4	6F3A	11.3	9.5	18.4	13.8
4	6F6B	11.4	1.7	18	1
4	7F6A1	11.1	19.2	17.2	19.3

While certain features of the invention have been illustrated and described herein, many modifications, substitutions, changes, and equivalents will now occur to those of ordinary skill in the art. It is, therefore, to be understood that the appended claims are
5 intended to cover all such modifications and changes as fall within the true spirit of the invention.

CLAIMS

1. An antibody that binds to human OSCAR and antagonizes OSCAR induced effects, wherein said antibody competes with antibody 7F1A1 for binding to OSCAR, wherein the amino acid sequence of the 7F1A1 heavy chain is as set forth in SEQ ID NO 6 and the amino acid sequence of the 7F1A1 light chain is as set forth in SEQ ID NO 5.

2. An antibody according to claim 1, wherein said antibody binds to the same epitope, or at least a portion of the same epitope, as the 7F1A1 antibody.

3. An antibody that binds to human OSCAR and antagonizes OSCAR induced effects, wherein said antibody competes with antibody 7F21A1 for binding to OSCAR, wherein the amino acid sequence of the 7F21A1 heavy chain is as set forth in SEQ ID NO 16 and the amino acid sequence of the 7F21A1 light chain is as set forth in SEQ ID NO 15.

4. An antibody according to claim 3, wherein said antibody binds to the same epitope, or at least a portion of the same epitope, as the 7F21A1 antibody.

5. An antibody that binds to human OSCAR and antagonizes OSCAR induced effects, wherein said antibody competes with antibody 3F7A9 for binding to OSCAR, wherein the amino acid sequence of the 3F7A9 antibody heavy chain is as set forth in SEQ ID NO 20 and the amino acid sequence of the 3F7A9 light chain is as set forth in SEQ ID NO 19.

6. An antibody according to claim 5, wherein said antibody binds to the same epitope, or at least a portion of the same epitope, as the 7F7A9 antibody.

7. An antibody according to any one of claims 1-6, wherein said antibody is not an R-phycoerythrin conjugated antibody.

8. An antibody according to any one of claims 1-7, wherein said antibody is not an 11.1CN5 antibody.

9. An antibody comprising the three CDR sequences as set forth in SEQ ID NO 6 and three CDR sequences as set forth in SEQ ID NO 5.

10. An antibody comprising the three CDR sequences as set forth in SEQ ID NO 16 and the three CDR sequences as set forth in SEQ ID NO 15.

11. An antibody according to any one of the preceding claims, wherein said antibody reduces collagen I and/or collagen II induced secretion of TNF-α and/or IL-8 in immature dendritic cells (DC).

12. An antibody according to any one of the preceding claims, wherein said antibody reduces collagen I induced secretion of IL-8 and/or TNF-alpha from synovial fluid mononuclear cells from rheumatoid arthritis (RA) patients.

13. A pharmaceutical composition comprising an antibody according to any one of claims 1-12.

14. A pharmaceutical composition according to claim 13, wherein no sodium azide has been added to said composition.

15. Use of an antibody according to any one of claims 1-12, or a pharmaceutical composition according to any one of claims 13-14, for treating an autoimmune inflammatory disease.

16. Use of an antibody according to any one of claims 1-12, or a pharmaceutical composition according to any one of claims claim 13-14, for treating RA.

17. A method for inducing collagen dependent maturation of monocyte-derived Dendritic Cells (moDCs), wherein said method comprises incubating moDCs on a surface of essentially neutral charge in the presence of collagen.

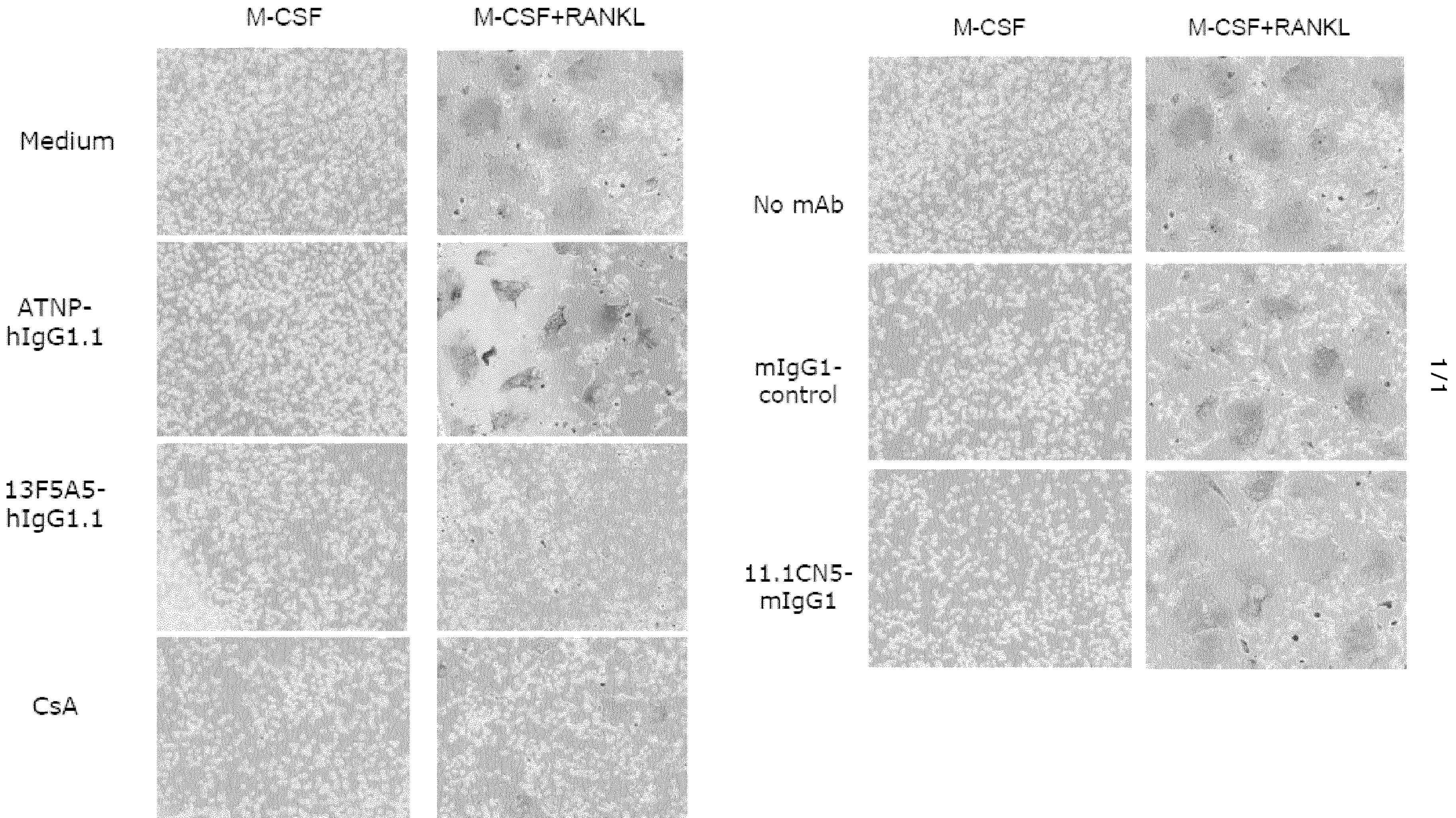

Fig. 1

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2012/064108

A. CLASSIFICATION OF SUBJECT MATTER
INV. C07K16/28
ADD. A61K39/395 A61P19/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

EPO-Internal, BIOSIS, EMBASE, WPI Data, Sequence Search

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2010/040998 A1 (CAMBRIDGE ENTERPRISE LIMITED) 15 April 2010 (2010-04-15) cited in the application	9,11-16
A	page 4, paragraph 4 page 11, paragraph 3 page 41, last paragraph - page 42, paragraph 1 page 44, paragraph 4 figure 4 -----	1,2,7,8

☐ Further documents are listed in the continuation of Box C.

☒ See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
15 August 2012	10/01/2013

Name and mailing address of the ISA/	Authorized officer
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1

INTERNATIONAL SEARCH REPORT

International application No.
PCT/EP2012/064108

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

1, 2, 9(completely); 7, 8, 11-16(partially)

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. claims: 1, 2, 9(completely); 7, 8, 11-16(partially)

   An antibody that competes with antibody 7F1A1 or binds to the same epitope on human OSCAR as antibody 7F1A1, and antagonizes OSCAR-induced effects. Pharmaceutical composition and therapeutic uses.

   ---

2. claims: 3, 4, 10(completely); 7, 8, 11-16(partially)

   An antibody that competes with antibody 7F21A1 or binds to the same epitope on human OSCAR as antibody 7F21A1, and antagonizes OSCAR-induced effects. Pharmaceutical composition and therapeutic uses.

   ---

3. claims: 5, 6(completely); 7, 8, 11-16(partially)

   An antibody that competes with antibody 3F7A9or binds to the same epitope on human OSCAR as antibody 3F7A9, and antagonizes OSCAR-induced effects. Pharmaceutical composition and therapeutic uses.

   ---

4. claim: 17

   Method for inducing collagen-dependent maturation of monocyte-derived dendritic cells (moDCs), wherein said method comprises incubating moDCs on a surface of essentially neutral charge in the presence of collagen.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/EP2012/064108

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2010040998 A1	15-04-2010	CA 2739679 A1	15-04-2010
		CN 102282169 A	14-12-2011
		EP 2344530 A1	20-07-2011
		US 2012028903 A1	02-02-2012
		WO 2010040998 A1	15-04-2010