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Antibodies as an unlimited source of anti-infective, anti-tumour and immunomodulatory peptides

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ABSTRACT

Antibodies (Abs) are emerging as an important class of therapeutic agents for the treatment of various human diseases, often conjugated to drugs or toxic substances. In recent years, the incidence of cancer and infectious diseases has increased dramatically, making it imperative to discover new effective therapeutic molecules. Among these, small peptides are arousing great interest. Synthetic peptides, representative of variable and constant region fragments of Abs, were proved to exert in vitro*,* ex vivo *and/or* in vivo *anti-microbial, anti-viral, anti-tumour and/or immunomodulatory activities, mediated by different mechanisms of action and regardless of the specificity and isotype of the Ab. Some of these synthetic peptides possess the ability to spontaneously and reversibly self-assemble in an organised network of fibril-like structure. Ab fragments may represent a novel model of targeted anti-infective and anti-tumour auto-delivering drugs.*

Keywords: *antibodies, anti-infective peptides, anti-tumour pepetides, immunomodulatory peptides*

1. Antibodies

Antibodies (Abs) or immunoglobulins (Igs) are homodimeric glycoproteins, organised in a Y-shaped quaternary structure, consisting of two identical light chains (L) of 250 amino acids (λ or κ), and two identical heavy chains (H) of about 450–550 amino acids (α, γ, δ, ε or μ), covalently linked by disulfide bridges. Each chain is composed of one variable (V_L and V_H) and one (C_L) or more (C_H) constant functional domains, containing an intra-chain disulfide bond, respectively involved in the recognition of the antigen (Ag) and in effector functions. Each V_L and V_H domain comprises three complementarity determining regions (CDRs; L_{1-3} and H_{1-3}), highly variable in sequence and length, responsible for Ab specificity[1]. Digestion with papain gives rise to a crystallisable fragment (Fc) and two Ag binding fragments (Fabs) that bind identical epitopes (antigenic determinants). Fc portion defines the isotype and influences not only Ab tissue distribution, placental transfer, and half-life in serum, activation of the complement pathway and differential binding to various cell types, but also antigen recognition or binding[2] (Figure 1).

Abs evolved in vertebrates as the sophisticated and versatile mechanism of host defence against pathogens. Their function was firstly reported in 1890, when Emil von Behring and Shibasaburo Kitasato successfully treated diphtheria and tetanus by transfer

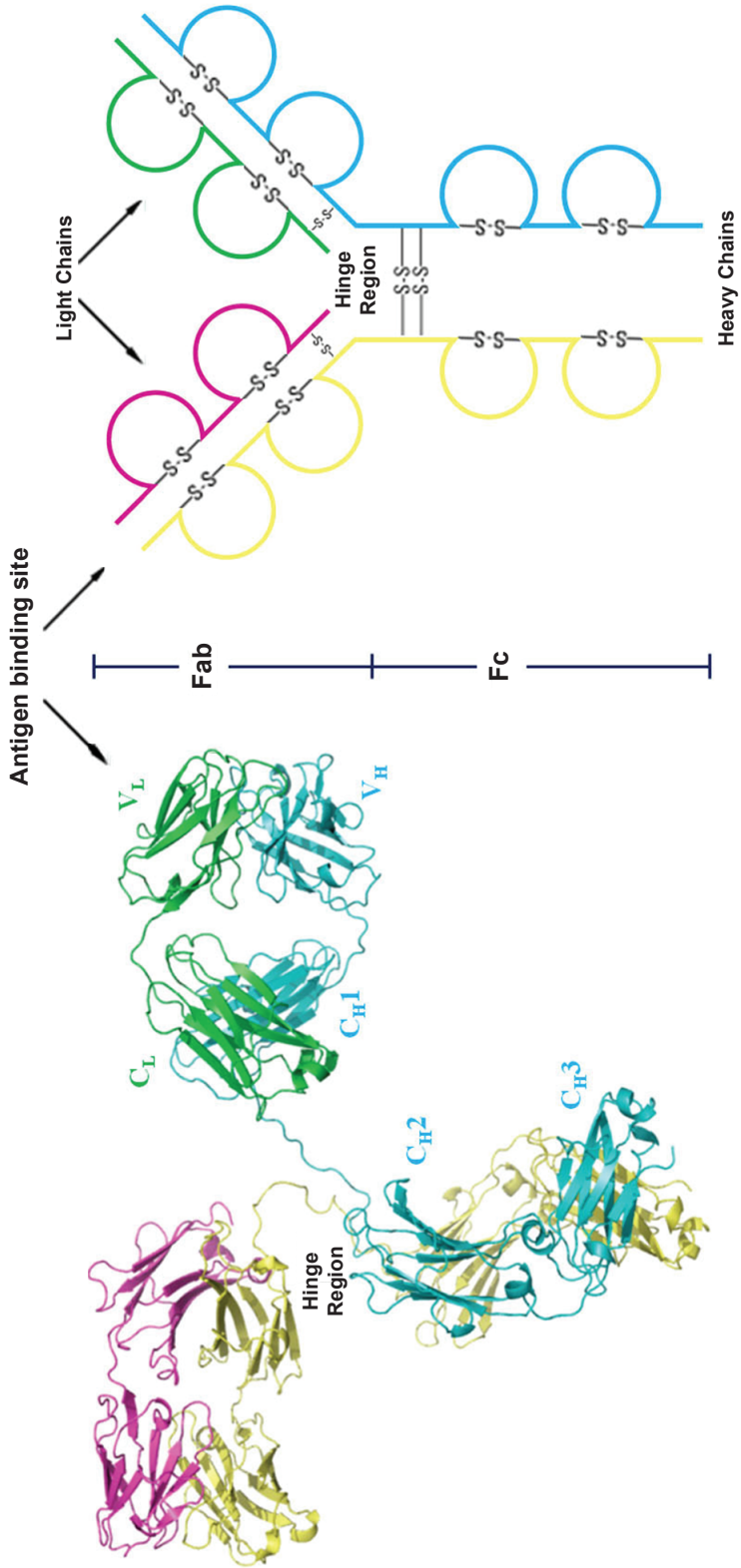

Figure 1 *Structural (left) and schematic (right) representation of a monomeric immunoglobulin.*

of serum from toxin-immunised to naïve animals[3]. The following year Paul Ehrlich used for the first time the term "Antikörper" (German word for "antibody")[4]. During the initial differentiation of B lymphocytes, Abs are expressed as membrane receptors on the cell surface and, as a result of specific stimuli, are secreted by plasma cells. Depending on the amount, specificity and isotype, Abs may play a significant role in immunoprotection, recognising and specifically binding to a large variety of protein and polysaccharide Ags. After Ag binding, Ab may be protecting by direct neutralisation of bacterial toxins or viruses, or by recruitment, through Fc portion, of other components of the immune system, such as complement, phagocytes and/or natural killer (NK) cells, events that can determine the outcome of host–pathogen interaction. Strategies for active (vaccines) and passive (sera) immunoprophylaxis are based on Abs involvement in anti-infective immunoprotection. Recently, Abs characterised by different properties have been discovered, in particular Abs endowed with catalytic activity (CAbs), named abzymes[5], or with direct microbicidal activity, named killer Abs or "antibiobodies" (KAbs)[6].

2. Antibodies as therapeutic agents

In recent decades, Abs emerged as a new class of therapeutic agents in different clinical settings, including oncology, autoimmune and cardiovascular diseases, infectious diseases, chronic inflammation and transplantation, due to their high selectivity and specificity in recognition of targeted molecules[7].

Polyclonal Abs, obtained from human donors or hyperimmune animals, are still used in medicine, for the neutralisation of viruses and bacterial toxins, replacement therapy in patients with Ig deficiency, or treatment of some medical emergencies, such as poisoning by snake or spider bites, digoxin, digitoxin and plant toxins, for which immediate immunoprotection is imperative.

The ability to produce monoclonal (mAbs) on an industrial scale led to a revival of therapies based on Abs. The available mAbs are defined using different suffixes, such as "umab" (human mAb), "momab" (mouse mAb), "ximab" (chimeric mAb) and "zumab" (humanised mAb).

In particular, the increasing knowledge of the major cellular pathways in the induction and progression of tumours, as well as in immunology, led to the approval by regulatory agencies of a number of native or engineered mAbs for the treatment of cancers, autoimmune diseases, transplant rejection and other clinical conditions, such as neurological and IgE-mediated disorders and chronic inflammatory diseases, *e.g.* anti-tumour necrosis factor (TNF)-α Ab in rheumatoid arthritis, Crohn's disease, and psoriasis (Table 1)[8]. Many other Abs are undergoing clinical evaluation.

Cancer therapy mediated by Abs is essentially based on Ag targets preferentially expressed on malignant cells. These targets may play important roles in signal transduction and ion transport or act as surface receptors. Ag recognition may lead to antibody-dependent cell-mediated cytotoxicity (ADCC) or complement-dependent cytotoxicity (CDC) responses. Abs may also induce the expression of chemokines that can attract neutrophils (PMNs), macrophages (MΦs) and NK cells. Angiogenic factors may also be tumour targets[9].

Table 1 *Approved mAbs for therapeutic use in various clinical conditions*

mAb	Trade name	Ab format	Indication	Antigen	Approval	Company
*Muromonab-CD3	Orthoclone OKT3	Murine IgG2aκ	Organ graft rejection	CD3	1986 (FDA, EMA)	Johnson & Johnson
Abciximab	ReoPro	Chimeric Fab of IgG1 7E3	Cardiovascular diseases	GPIIb/IIIa receptor	1994 (FDA, EMA)	Johnson & Johnson
Rituximab	Rituxan	Chimeric IgG1κ	Non-Hodgkin's lymphoma, chronic lymphocytic leukaemia and rheumatoid arthritis	CD20	1997 (FDA) 1998 (EMA)	Genentech and Biogen Idec
*Daclizumab	Zenapax	Humanised IgG1κ	Prophylaxis of acute organ rejection in renal transplants	IL-2Rα (CD25)	1997 (FDA) 1999 (EMA)	Roche
Basiliximab	Simulect	Chimeric IgG1κ	Prophylaxis of acute organ rejection in renal transplants	IL-2Rα (CD25)	1998 (FDA, EMA)	Novartis
Infliximab	Remicade	Chimeric IgG1κ	Crohn's disease and rheumatoid arthritis	TNF-α	1998 (FDA) 1999 (EMA)	Johnson & Johnson
Trastuzumab	Herceptin	Humanised IgG1κ	Breast cancer	HER2 (CD340)	1998 (FDA) 2000 (EMA)	Genentech/Roche
*Gemtuzumab ozogamicin	Mylotarg	Calicheamicin-humanised IgG4κ	Acute myeloid leukaemia	CD33	2000 (FDA)	Wyeth/Pfizer
Alemtuzumab	Campath	Humanised IgG1κ	B-cell chronic lymphocytic leukaemia	CD52	2001 (FDA, EMA)	Genzyme
Ibritumomab tiuxetan	Zevalin	^{90}Y-murine IgG1κ	B-cell non-Hodgkin's lymphoma	CD20	2002 (FDA) 2004 (EMA)	Biogen Idec
Adalimumab	Humira	Human IgG1κ	Rheumatoid arthritis and Crohn's disease	TNF-α	2002 (FDA) 2003 (EMA)	Abbott
Omalizumab	Xolair	Humanised IgG1κ	Moderate to severe persistent asthma	IgE	2003 (FDA) 2005 (EMA)	Genentech/Roche
Tositumomab	Bexxar	^{131}I-murine IgG2aλ	Non-Hodgkin's lymphoma	CD20	2003 (FDA)	Corixa/GSK
*Efalizumab	Raptiva	Humanised IgG1κ	Moderate to severe plaque psoriasis	CD11a	2003 (FDA) 2004 (EMA)	Genentech/Roche

Table 1 continued

mAb	Trade name	Ab format	Indication	Antigen	Approval	Company
Cetuximab	Erbitux	Chimeric IgG1κ	Colorectal cancer, head and neck cancer	EGFR	2004 (FDA, EMA)	ImClone/BMS/ Merck kGa
Bevacizumab	Avastin	Humanised IgG1κ	Various solid tumours	VEGF-A	2004 (FDA) 2005 (EMA)	Genentech/Roche
Natalizumab	Tysabri	Humanised IgG4κ	Multiple sclerosis and Crohn's disease	α4-integrin	2004 (FDA) 2006 (EMA)	Biogen Idec
Ranibizumab	Lucentis	Humanised Fab	Age-related macular degeneration	VEGF-A	2006 (FDA) 2007 (EMA)	Genentech/Roche
Panitumumab	Vectibix	Human IgG2κ	Metastatic colorectal carcinoma	EGFR	2006 (FDA) 2007 (EMA)	Amgen
Eculizumab	Soliris	Humanised IgG2/4κ	Paroxysmal nocturnal haemoglobinuria	C5	2007 (FDA, EMA)	Alexion
Certolizumab pegol	Cimzia	Pegylated humanised Fab	Rheumatoid arthritis and Crohn's disease	TNF-α	2008 (FDA) 2009 (EMA)	UCB
Golimumab	Simponi	Human IgG1κ	Rheumatoid arthritis, psoriatic arthritis and ankylosing spondylitis	TNF-α	2009 (FDA, EMA)	Johnson & Johnson
Canakinumab	Ilaris	Human IgG1κ	Cryopyrin-associated periodic syndromes	IL-1β	2009 (FDA, EMA)	Novartis
Ustekinumab	Stelara	Human IgG1κ	Plaque psoriasis	IL-12/IL-23	2009 (FDA, EMA)	Johnson & Johnson
Ofatumumab	Arzerra	Human IgG1κ	Chronic lymphocytic leukaemia	CD20	2009 (EMA)	Genmab
Tocilizumab	Actemra	Humanised IgG1κ	Rheumatoid arthritis	IL-6R (CD126)	2010 (FDA)	Chugai/Roche
Denosumab	Prolia	Human IgG2κ	Prevention of cancer skeleton-related events	RANKL	2010 (FDA)	Amgen
Catumaxomab	Removab	Murine/rat hybrid IgG	Intraperitoneal treatment of malignant ascites in patients with EpCAM-positive carcinomas	EpCAM and CD3	2009 (EMA)	TRION Pharma
Belimumab	Benlysta	Human IgG1λ	Systemic lupus erythematosus	BLyS	2011 (FDA)	Human Genome Sciences

Table 1 continued

mAb	Trade name	Ab format	Indication	Antigen	Approval	Company
Ipilimumab	Yervoy	Human IgG1κ	Melanoma	CTLA-4 (CD152)	2011 (FDA)	BMS
Brentuximab vedotin	Adcetris	Vedotin-chimeric IgG1κ	Relapsed or refractory Hodgkin lymphoma and systemic anaplastic large cell lymphoma	CD30	2011 (FDA) 2012 (EMA)	Seattle Genetics
Pertuzumab	Perjeta	Humanised IgG1κ	Breast cancer	HER2 (CD340)	2012 (FDA)	Genentech/Roche
Trastuzumab-DM1	T-DM1	DM1 attached to trastuzumab	Breast cancer	HER2 (CD340)	2013 (FDA)	Genentech/Roche
Obinutuzumab	Gazyva	Humanised and glyco-engineered IgG	Chronic lymphocytic leukaemia	CD20	2013 (FDA)	Genentech/Roche

*Withdrawn by the sponsor.

CD, cluster of differentiation; FDA, Food and Drug Administration; EMA, European Medicines Agency; HER2, human epidermal growth factor receptor 2; ^{90}Y, yttrium-90; ^{131}I, iodine-131; EGFR, epidermal growth factor receptor; VEGF-A, vascular endothelial growth factor A; C5, complement protein C5; IL, interleukin; RANKL, receptor activator of nuclear factor kappa-B ligand; EpCAM, epithelial cell adhesion molecule; BLyS, B-lymphocyte stimulator; CTLA-4, cytotoxic T-lymphocyte antigen 4; DM1, mertansine.

Table 2 *Approved mAbs for therapeutic use in infectious diseases*

mAb	Trade Name	Ab format	Indication	Antigen	Approval	Company
Palivizumab	Synagis	Humanised IgG1κ	Respiratory syncytial virus (RSV) infection	RSV F	1998 (FDA) 1999 (EMA)	MedImmune
Raxibacumab	ABThrax	Human IgG1λ	Prophylaxis and treatment of anthrax	*B. anthracis* PA	2012 (FDA)	GlaxoSmithKline
Motavizumab	Numax	Humanised IgG1κ	RSV infection	RSV F	Pending	MedImmune
Efungumab	Mycograb	Human scFv	Invasive *Candida* infection	Fungal Hsp90	Authorisation refused (EMA)	NeuTec Pharma

RSV F, human respiratory syncytial virus glycoprotein F; PA, protective antigen; scFv, single-chain variable fragment; Hsp90, heat shock protein 90.

A few mAbs have been approved for clinical use in the field of infectious diseases (Table 2), many others are currently undergoing pre-clinical and clinical development, or their experimental use has been described in the literature. In recent years, however, the dramatic increase of new viral diseases, life-threatening fungal infections, threats of bioterrorism (*e.g.*, anthrax) and bacterial infections caused by strains/species resistant to antibiotics (*e.g.,* methicillin-resistant *Staphylococcus*), have renewed the interest in anti-infective therapy based on Abs[10,11].

To improve the therapeutic activity, Abs have been modified to convey toxic substances, engineered to bind multiple epitopes (bi-specific Abs) or even to acquire new catalytic activities (abzymes). Direct conjugation to anti-microbial drugs or other cytotoxic agents (Ab drug conjugates, ADCs) may increase the clinical efficacy with fewer side effects[12–14]. New immunotherapy approaches are based on the concomitant administration of different Abs, often in combination with conventional chemotherapy, cytokines or growth factors[15].

3. Antibody-derived bioactive peptides

The development of innovative methods of genetic engineering allowed the production of recombinant Abs (rAbs) and the obtaining of synthetic Ab fragments of lower molecular weight, characterised by their simple genetic manipulation and relatively low cost, opening new avenues for the potential use of pharmacologically active Ab-derived peptides for the treatment of infectious diseases, cancer, neurological and immune disorders (Table 3).

4. Anti-infective killer peptides

According to the idiotypic network theory formulated by Jerne in 1974[31], in the course of an immune response against an Ag, specific Abs are produced, which in turn stimulate the production of anti-idiotypic (anti-Id) Abs, that may represent the internal image of the Ag and even mimic its biological activity. Through the approach of the Id network it was possible to reproduce the broad-spectrum anti-microbial action of a killer toxin of *Wickerhamomyces anomalus* (formerly *Pichia anomala*) ATCC 96603 (*Pa*KT), by the production of anti-Id Abs (KAbs)[6]. Such Abs have been shown to possess anti-microbial activity *in vitro* and *in vivo* against pathogenic microorganisms taxonomically unrelated, including drug-resistant strains[32].

In an attempt to establish the possible correlation between the amino acid sequence of an anti-Id rKAb (scFv H6) and its candidacidal activity, a number of peptides pertaining to the hypervariable region, including the six CDRs, were synthesised and tested *in vitro* against *C. albicans*, selected as a sensitive model microorganism[33]. A decapeptide, called P6 (EKVTMTCSAS), having the first three amino acids of the CDR L_1 (SAS) in the carboxy (C)-terminal position, showed the highest candidacidal activity. A derivative thereof obtained by substitution of the first residue with alanine, referred to as killer peptide (KP, AKVTMTCSAS), demonstrated a differential *in vitro*, *in vivo*, *in planta*, and/or *ex vivo* anti-microbial and anti-viral activity (Table 4).

Surprisingly, P6 sequence was also present in other Vκ of unrelated Abs with different specificities. As a proof of the concept of the intrinsic biological potential of Ab fragments, CDR peptides derived from well characterised murine and human mAbs were

Table 3 *Ab-derived peptides characterised by anti-infective, immunomodulatory, and/or anti-tumour properties*

Peptide	Origin (aa)	Sequence	Activity	Target
Tuftsin[16]	IgG C_H2 domain (289–292)	TKPR	Stimulation of phagocytising cells	MΦs, PMNs and NK cells
Rigin[17]	IgG C_H3 domain (341–344)	GQPR	*In vitro* phagocytosis against *S. aureus* and production of IL-1 and TNF-α by monocytes	Monocytes/MΦs
p24[18]	IgG1 Fc fragment (335–358)	TISKAKGQPREPQVYTLPPSRDEL	Proliferation of B-cells through production of growth factors by T-helper cells	B-cells
Immunocortin[19]	IgG V_H domain (11–20)	VKKPGSSVKV	Immunosuppressive effect *in vitro* and some neurotrophic features	Thymocytes and peripheral MΦs
Immunorphin[20]	IgG C_H3 domain (364–373)	SLTCLVKGFY	Influence on immune, neuroendocrine and cardiovascular systems	Non-opioid receptor of β-endorphin
H3[21]	CDR H_3 of AC7	RQMIRGYFDV	Inhibition of platelet aggregation and fibrinogen binding to platelet	Fibrinogen
PEP3H[22]	CDR H_3 of RS-348	ARDPDYYDNYFYAMDYWGPGT	*In vitro* and *in vivo* neutralisation of the RSV virus	RSV F
CM2 CM9 CM11[23]	V_H ST40 V_L^L ST40 V_L ST40	KCSGYGFTNAGMQWCK KCDSYMNWYQQKPGCK KCWTFGGGTYLEIKCK	Inhibition of HIV-1 promoter activation	CD4
AHNP[24]	Functional mimetic of trastuzumab	YCDGFYACYMDV	*In vitro* and *in vivo* anti-tumour activity	$p185^{HER2/neu}$
GLYX-13[25]	CDR L_3 of B6B21	TPPT-amide	NMDA receptor modulator that potentiates learning	NMDAR
F58[26]	CDR H_3 of F58	CDLIYYDYEEDYYFDYC	Neutralisation of HIV-1 IIIB	gp120-V3 loop
pE51[27]	CDR H_3 of E51	NSIAGVAAAGDYADYDGGYYYDMD	Neutralisation of HIV-1	gp120
Pheromonicins[28]	Colicin 1a-CDR H_1-FR H_2-CDR L_3		*In vitro* and *in vivo* anti-tumour activity	Tumour-specific surface markers
hCDR1[29]	CDR H_1 of human anti-DNA autoAb	GYYWSWIRQPPGKGEEWIG	Specific treatment of systemic lupus erythematosus	MHC-II
EPPT[30]	mAb ASM2 CDR H_3-derived	EPPT	*In vitro* and *in vivo* tumour targeting	Breast cancer cells

aa, amino acids position; HIV, human immunodeficiency virus; NMDAR, N-methyl-D-aspartate receptor; gp120, glycoprotein 120; MHC-II, major histocompatibility complex class II.

Table 4 *Multifunctional biological activities of KP*

Pathogen	Activity	Description
C. albicans[33] (including drug-resistant strains)	*in vitro*	Fungicidal activity [μM] inhibited by β-1,3-D-glucans in a dose-dependent way
	in vivo	Therapeutic effect against mucosal and systemic candidiasis in immunocompetent and SCID animals
Candida spp.[34] (including drug-resistant strains)	*in vitro*	Potent fungicidal activity
Cryptococcus neoformans[35]	*in vitro*	Impaired production of specific virulence factors
	in vivo	Therapeutic effect against systemic cryptococcosis in immunosuppressed mice and reduction of fungal burden in brain
Paracoccidioides brasiliensis[36]	*in vitro*	Significant anti-fungal activity
	in vivo	Therapeutic effect against experimental paracoccidioidomycosis and reduction of the fungal load in lung, spleen, and liver
Saccharomyces cerevisiae[37]	*in vitro*	Fungicidal activity against all the defective mutants of a wide *S. cerevisiae* gene deletion strain collection
Malassezia pachydermatis[38]	*in vitro*	Fungicidal activity
	in vivo	Therapeutic effect against *Malassezia* otitis in dogs
Pseudomonas syringae, *Erwinia carotovora*, *Botrytis cinerea* and *Fusarium oxysporum*[39]	*in vitro* and *in planta*	Bacterial and fungicidal activity
Leishmania major, *L. infantum*[40]	*in vitro*	Anti-proliferative and leishmanicidal activity
Acanthamoeba castellanii[41]	*in vitro*	Killing activity
HIV-1[42]	*in vitro*	Inhibition of viral replication of X4 and R5 strains [μM]
	ex vivo	Down-regulation of CCR5 cell surface expression
Avian (H7N1 and H7N3) and human (H1N1 and H3N2 amantadine-resistant) influenza viruses[43]	*in vitro*	Reduction of late proteins M1 and HA
	in vivo	Improved survival of mice and decreased pulmonary virus titers

[μM], micromolar concentration; SCID, severe combined immunodeficiency; X4, lymphocytotropic HIV strain; R5, monocytotropic HIV strain; CCR5, C–C chemokine receptor type 5; M1, matrix protein; HA, haemagglutinin.

synthesised. In particular, mAb C7, a murine IgM generated against the main target of secretory IgA in the cell wall of *C. albicans*, was selected for its proven anti-fungal and anti-tumour activities[44,45]. Murine mAb pc42, an IgM directed to the dominant epitope within a synthetic peptide model antigen including B and T-helper lymphocyte epitopes, was selected for sharing CDRs H_1 and H_2 with mAb C7. Human mAb HuA, an IgM specific for the most frequent difucosylated human blood group A substance, devoid of any homology with CDRs of mAbs C7 or pc42, was selected to represent Abs widespread in the population. The biological activity of all the CDRs was evaluated in several systems. Regardless of the native Ab specificity, synthetic CDRs showed differential anti-microbial and anti-viral effects *in vitro*, *ex vivo* and/or *in vivo*, in a manner reminiscent of the molecules of innate immunity (Table 5)[46].

Subsequently, peptides belonging to the constant region (Fc peptides) of human Abs of different isotype (IgG1, IgM, IgA1), were selected by using a bioinformatic approach. Some of them proved to have important biological properties against a variety of pathogenic yeasts including strains resistant to conventional anti-fungal drugs (Table 6)[47].

Table 5 *Biological properties of selected CDR-derived peptides*

CDR	mAb	Peptide sequence	Pathogen	Activity	Description
L_1	C7	KSSQSLLNSGNQKNYLT	*C. albicans*	*in vitro*	Fungicidal activity
				in vivo	Therapeutic effect against systemic candidiasis
L_2	C7	WASTRES	HIV-1 (R5)	*in vitro*	Inhibition of viral replication
L_3	C7	NDYSYPRSR	*C. albicans*	*in vitro*	Fungicidal activity
H_1	C7/pc42	GYYMH	HIV-1 (R5)	*ex vivo*	Inhibition of viral replication
H_2	C7/pc42	YISCYNGATSYNQKFK	*C. albicans*	*in vitro*	Fungicidal activity
H_3	C7	ARQGVRGGAMD	HIV-1 (X4)	*in vitro*	Inhibition of viral replication
			C. albicans	*in vitro*	Fungicidal activity
L_1	pc42	YRASKSVSTSGYSYMH	HIV-1 (R5)	*ex vivo*	Inhibition of viral replication; Peptide motif is present in REV
L_2	pc42	LVSNLES	*C. albicans*	*in vitro*	Fungicidal activity
			C. albicans	*in vitro*	Fungicidal activity
L_3	HuA	QQRSNWPRS	HIV-1 (R5)	*in vitro/ ex vivo*	Inhibition of viral replication; Peptide motif is present in RT

REV, regulator of expression of virion protein; RT, reverse transcriptase protein.

Table 6 *Anti-fungal activity of selected Fc-derived peptides*

Peptide	Origin (aa)	Sequence*	Activity	Description
H4L	IgG1 C_{H3} (312–315) IgM C_{H4} (418–421) IgA1 C_{H3} (317–320)	HEAL	*in vitro*	Weak anti-fungal activity
N10K	IgG1 C_{H3} (244–253)	NQVSLTCLVK	*in vitro*	Anti-fungal activity
			in vivo	Therapeutic effect against mucosal and systemic candidiasis
T11F	IgM C_{H2} (197–207)	TCRVDHRGLTF	*in vitro*	Anti-fungal activity

aa, amino acids position; *PIR AC: P01857(IgG1), P01871(IgM), P01876 (IgA1).

Moreover, the alanine substituted derivatives (asds) of N10K and T11F, the two most active Fc peptides, showed increased, unchanged, or decreased anti-fungal activity. In particular, the replacement of cysteine residues, involved in the formation of disulfide bridges and, to a lesser extent, the replacement of positively charged residues resulted in a significant loss of candidacidal activity. In contrast, the replacement of the only negatively charged residue of T11F peptide resulted in an increase of candidacidal activity[47].

5. Anti-tumour peptides

Over the years, different Ab-derived peptides characterised by *in vitro* and/or *in vivo* anti-tumour activity have been identified. In particular, C7/pc42 CDR H_2 and HuA CDR L_1 proved to have direct anti-tumour activity *in vitro* against several tumour cell lines. Both peptides caused caspase-dependent apoptosis in melanoma B16F10-Nex2 and leukaemic HL-60 cells, reducing the growth of melanoma. C7/pc42 H_2 contains an amphipathic β region in C-terminal position (SYNQKFK) responsible for the binding to the β-actin

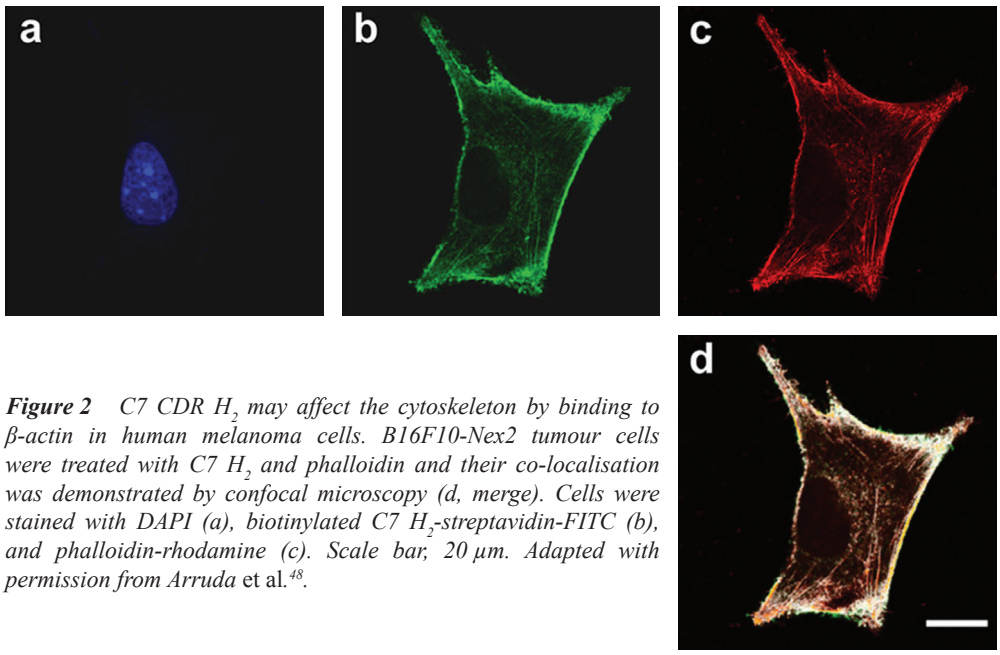

Figure 2 *C7 CDR H_2 may affect the cytoskeleton by binding to β-actin in human melanoma cells. B16F10-Nex2 tumour cells were treated with C7 H_2 and phalloidin and their co-localisation was demonstrated by confocal microscopy (d, merge). Cells were stained with DAPI (a), biotinylated C7 H_2-streptavidin-FITC (b), and phalloidin-rhodamine (c). Scale bar, 20 µm. Adapted with permission from Arruda* et al.[48].

receptor on the surface of melanoma cells, inducing β-actin polymerisation and F-actin stabilisation (Figure 2), formation of reactive oxygen species and apoptosis.

C7/pc42 H_2 have been shown to inhibit the formation of lung metastases, being devoid of toxicity, both *in vitro* and *in vivo*, against non-cancer cells. C7 CDRs H_2, L_1, L_2 and L_3 at higher concentrations inhibited specifically the germination of human umbilical vein endothelial cells (HUVEC)[46,48].

More recently, synthetic peptides representing the CDRs of two anti-melanoma murine mAbs (A4, an IgG2aκ, and A4M, an IgM) showed anti-tumour activity. A4 and A4M reacted differently and strongly with melanoma cells by recognising protocadherin β13 and histone 1, respectively. They induced apoptosis and inhibited tumour growth and metastasis *in vivo*. A4 H_3 was cytotoxic *in vitro* against melanoma cells by inducing apoptosis and DNA degradation and inhibited cell proliferation. A cyclic derivative thereof proved to be even more active. A4M L_1 and L_2 were found to inhibit tumour cell growth, trigger apoptosis (also in HL-60 leukaemia cells) and significantly inhibit endothelial cell sprouting (angiogenesis), thus suggesting their potential as emerging candidates for development as drugs for anti-melanoma therapy[49].

6. Immunomodulatory peptides

In addition to anti-microbial, anti-viral and anti-cancer activity, Ab-derived fragments were also shown to possess immunomodulatory properties. Since the discovery in 1970 of tuftsin, the first IgG-derived peptide (TKPR) characterised by immunomodulatory activities (mainly stimulation of phagocytising cells, first of all macrophages), it was assumed that functional proteins could be a source of biologically active peptides[50]. Recently, it was shown that KP peptide is able to bind selectively, in a way dose- and time-dependent, to murine dendritic cells (DCs) and, to a lesser extent, to MΦs. The

binding is mediated by specific cell surface receptors, such as MHC-II, CD16/32, specific ICAM-3 grabbing non-integrin (Sign-R1) molecules and type C lectins. The treatment with KP induced functional and phenotypic changes on DCs, negatively regulating the expression of MHC-I, MHC-II and co-stimulatory CD86, and positively that of CD8-α, CD80, CD40, improving their ability to activate and/or modulate T-lymphocyte reactivity and proliferation. CD8-α contributed to the production of IL-12, important cytokine to promote a T_h1 immune response against different pathogens, while CD80 appeared to stimulate the mechanisms of immune tolerance[51].

Interesting results derived from the study of synthetic peptides corresponding to the CDRs of murine (MoA) and human (HuA) mAbs specific for the difucosylated human blood group A substance. These mAbs, sharing no CDR homology, represent the different ways by which the immune system can recognise the same epitope. In particular, HuA CDR L_3 proved to possess candidacidal activity *in vitro* and was able to stimulate the production of IL-6 but not of TNF-α in murine splenocytes and peritoneal macrophages (PMs), while MoA CDR H_3 induced a significant increase of both, justifying its *in vivo* therapeutic effect against experimental systemic candidiasis, despite the absence of *in vitro* candidacidal activity. Indeed, PMs, but not PMNs, very rapidly took up MoA H_3 and this uptake induced Akt activation that finally led to phosphorylation of IkBα with consequent translocation of NF-kB into the nucleus. These molecular events were responsible for cellular activation and subsequent transcription of genes coding for proinflammatory cytokines such as TNF-α, a positive regulator of TLR-4 expression (Figure 3)[52]. The antigenic structures of the opportunistic fungus *C. albicans* are recognised by TLR-4. Thus, natural immune cells were activated by MoA H_3 interaction, increasing their ability to ingest and kill yeast cells. Additionally, increased TLR-4 expression on PMs could facilitate fungal recognition with consequently more prompt and efficient immune response.

Similarly, N10K peptide was shown to possess *in vitro* immunomodulatory activity in human monocytes. This process began with N10K internalisation in monocytes, to continue with hyper-expression of Dectin-1, the main receptor of yeast parietal β-glucans. N10K treatment caused increased β-glucan-induced activation of IkBα and Syk through their phosphorylation and CARD9 and an increase in the production of pro-inflammatory cytokines, such as IL-6, IL-12p40, IL-1β and TNF-α. This activation enhanced phagocytosis of non-opsonised *C. albicans* cells by monocytes (Figure 4)[53].

Demonstration of anti-microbial and immunomodulatory properties of Ab-derived peptides could open a new scenario on the interactions between innate and acquired immunity.

7. Structure–function relationship

Structure–function relationship of Ab-derived peptides was investigated by circular dicroism (CD). In particular, KP peptide proved to form an active dimer in non-reducing conditions[54]. The distribution along the sequence of hydroxylated amino acids intercalated with hydrophobic residues has allowed the formation of an amphipathic β strand.

KP dimers showed the ability to interact with other dimers and spontaneously and reversibly self-aggregate, in a process concentration- and temperature-dependent, to form a β-sheet structure. Formation of hydrogel-like aggregates was catalysed by glucans,

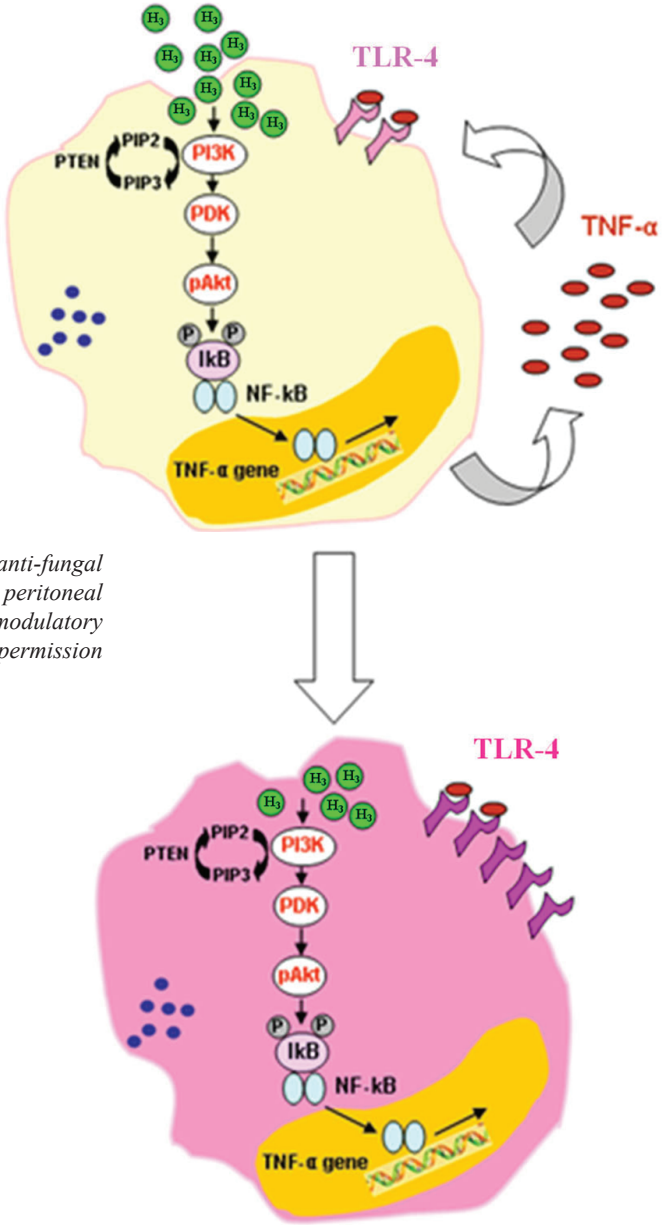

Figure 3 *Mechanism of anti-fungal protection induced in murine peritoneal macrophages by immunomodulatory MoA CDR H_3. Adapted with permission from Gabrielli* et al.[52].

soluble or exposed on the surface of *C. albicans* cells (Figure 5). Thus KP was proved to interact preferentially with laminarin, a β-1,3-D-glucan with little branched linear structure, through the formation of numerous hydrogen bonds between the hydroxyl groups (OH). The biologically inactive scramble peptide (SP, MSTAVSKCAT), in contrast, retained a random coil conformation even after months from the solubilisation. The hydrogel-like formation helped to provide protection against proteases and to ensure a slow release of the active peptide over time, while the affinity for β-glucans was responsible for its targeted activities[54].

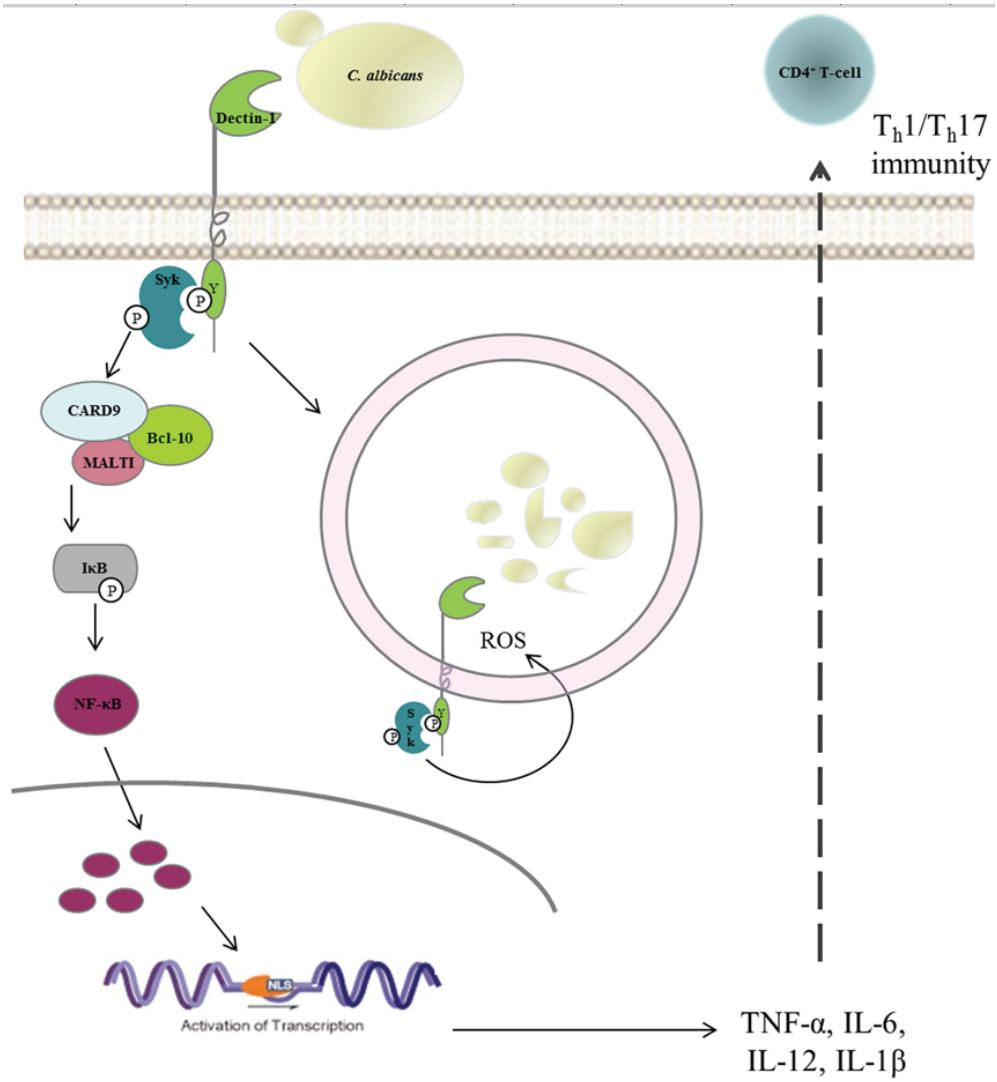

Figure 4 *Activation of phagocytosis of* C. albicans *cells induced in monocytes by Fc peptide N10K.*

Similarly to KP peptide, CD studies demonstrated the spontaneous aggregation over time of N10K peptide in fibrillar structures, going in just nine hours from a typical random coil conformation to a rich β-sheet structure. These observations were confirmed by TEM studies (Figure 6)[47]. The alternation of hydrophobic and hydrophilic residues in the sequence of N10K peptide was a prerequisite for the acquisition of a β-sheet structure and the consequent formation of larger aggregates. The presence of a greater number of hydrophobic residues in the N10K peptide could explain the greater rate and shorter time of aggregation compared to that of KP.

The reversible self-assembly, therefore, might represent a new paradigm of therapeutic autodelivering peptides[55].

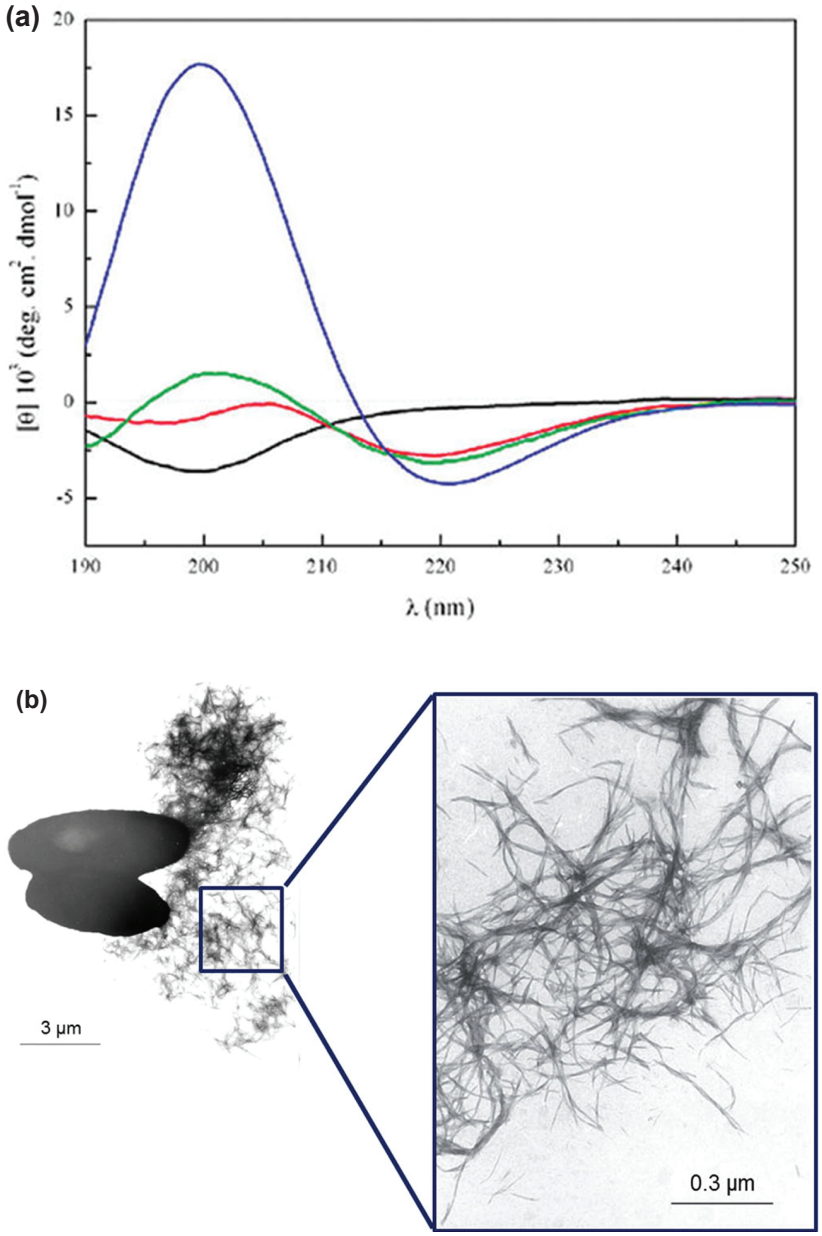

Figure 5 *Self-aggregation of KP peptide was investigated by circular dicroism (CD) spectroscopy and transmission electron microscopy (TEM). (a) KP was in random coil conformation 1 hour after its solubilisation in water (black line). With time, progressively the active dimers were structured to a β-sheet conformation (3 days, red line; 1 month, green line; and 6 months, blue line). (b) Cells of C. albicans were added to a freshly prepared aqueous solution of KP. Electron micrograph shows the instant formation of fibril-like structures highlighted in magnification. Adapted with permission from Pertinhez* et al.[54]*. Copyright (2009) American Chemical Society.*

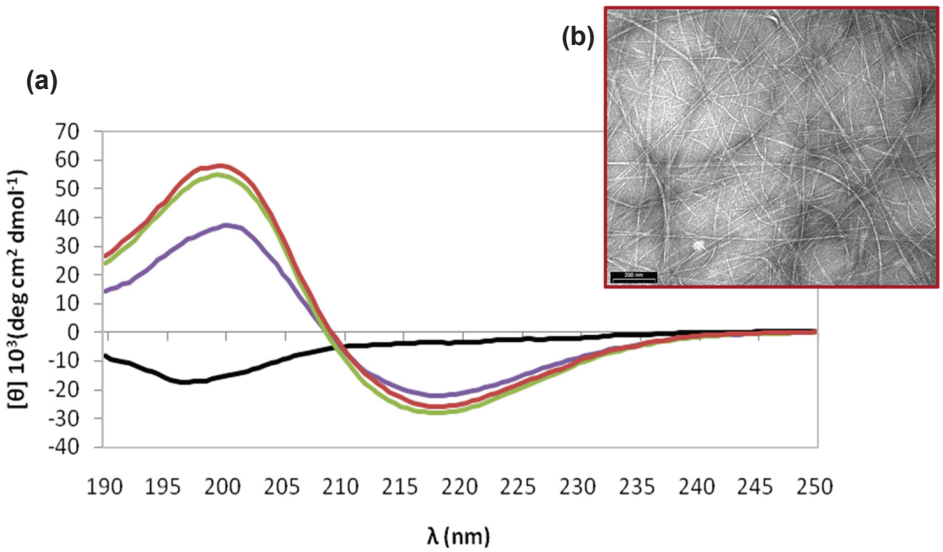

Figure 6 *Conformational transition of the IgG1 Fc-derived N10K peptide. (a) Far-UV CD spectra of N10K in water acquired at 20 °C. Self-aggregation was recorded as a function of time. N10K was in random coil conformation at 1 hour after solubilisation (black line) and quickly evolved to β-sheet structure already after 20 hours (purple line) to stabilise after 6 days (green line). 20 days from water solubilisation, CD spectrum (red line) and TEM micrograph (b) showed the formation of fibril-like structures. Adapted with permission from Polonelli* et al.[47].

7. Conclusions

In spite of the considerable technological progress and the continuous evolution of knowledge in the medical field, some diseases are still characterised by high morbidity and mortality rates. In the last decades, there has been a dramatic increase of patients with a variety of cancers, and an ever-increasing number of immunocompromised individuals accompanied by a significant growing of opportunistic infections, in particular of fungal origin. The massive and sometimes inappropriate use of drugs has contributed to the aggravation of the situation, encouraging the spread of strains resistant to anti-microbial drugs. Even today the infectious diseases are a global health problem and alternative control strategies based on conceptually innovative rational assumptions are needed[56].

In recent years, Abs in different formats increasingly emerged as therapeutic tools in several human diseases. Moreover, conjugation with conventional drugs, radioisotopes or other cytotoxic compounds, combined with better understanding of receptor–ligand interactions, enhanced their therapeutic activity, reducing the systemic side effects, in particular in tumour diseases and immunological disorders.

The description of microbicide Abs capable of killing a broad spectrum of pathogenic microorganisms through interaction with specific receptors of the cell wall (such as β-1,3-D-glucans or glucan-like molecules) absent in mammalian cells allowed to hypothesise the availability of new conceptually innovative therapeutic tools against various microbial infections, even in immunocompromised patients[57].

In this context, Ab fragments of different origin may represent intriguing innovative therapeutic tools by acting as cytotoxic and immunomodulatory molecules other than effectors of the adaptive immune response.

Recent advances in the development, administration, engineering and chemical optimisation of these peptides can positively affect selectivity, efficacy, bioactivity, stability to proteolysis, pharmacokinetics and/or pharmacodynamics, thus enhancing their therapeutic activity.

Is it possible that physiological proteolysis of Igs, beyond their half-life, may result in the release of Ab fragments with anti-microbial, anti-tumour and/or immunomodulatory activity? This hypothesis seems to be very challenging and might partly explain the apparent redundancy in the production of Abs. The proteolytic release of Ig-derived active fragments is possibly reminiscent of the intrinsic activity of other peptides derived from larger proteins[58,59].

The high frequency of Ab bioactive fragments suggest that Igs may represent a virtually unlimited source of potentially active peptides to be used for the development of new anti-infective, immunomodulatory and anti-cancer agents[60].

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