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Review

Natural IgM antibodies: from parias to parvenus

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Summary. Over the years, natural IgM antibodies were considered as the parias among the immune competent molecules. Their characteristic properties, like low affinity, cross-reactivity and pentameric structure, were assessed as difficult and nebulous. Today, mainly based on the persistent work of a few researchers and the key discoveries on innate immunity, natural IgM antibodies are "back on stage". Their important role in the immune response against invasive particles, modified self-components and altered cells is accepted. All the so far negatively judged features have to be seen in a different light, e.g. low affinity seems to be good for function and does not exclude specificity, cross-reactivity is no longer judged as unspecific, but instead as a very economic way of immune recognition and the pentameric structure is important for binding capacity and functional activities. In addition, with the use of natural IgM antibodies, a new field of tumour-specific targets has been encountered, the carbo-neo-epitopes, which are commonly found on post-transscriptionally modified membrane receptors. Having understood the typical features of natural IgM antibodies, their renaissance opens a new area of cancer therapeutics and diagnostics.

Key words: Natural IgM antibodies, Human antibodies, Immune surveillance, Malignancy

The immune surveillance system

Already 100 years ago, Paul Ehrlich predicted that the immune system not only eliminates microbacterial invaders, but also represses the growth of carcinomas, by providing antibodies against malignant cells (Ehrlich, 1902). Decades after Ehrlich it was Burnet who defined the immune surveillance concept as follows: "It is an evolutionary necessity that there should be some mechanisms for eliminating or inactivating such potentially dangerous mutant cells" (Burnet, 1964, 1970; Thomas, 1959, 1982).

To eliminate transformed cells nature has created an effective immune surveillance program consisting of an inherited, immediate response and a secondary immunity covering memory (Milner et al., 2005; Karin et al., 2006). The innate immunity is not only the first line of defense, but also the stimulus for the secondary acquired immune response (Hoebe et al., 2004). It consists of natural killer (NK) cells, dendritic and mast cells, macrophages and natural IgM antibody producing B cells (Bohn, 1999; MacPherson et al., 1999; Boes, 2000; Ochsenbein and Zinkernagel, 2000; Martin and Kearney, 2001; Parkin and Cohen, 2001; Moretta et al., 2002; Zitvogel, 2002). This system can distinguish between self and non-self and is responsible for the first specific immune response directed against bacteria, viruses and malignant cells (Greenberg and Grinstein, 2000; Mekori and Metcalfe, 2000; Moretta et al., 2002; Dunn et al., 2004; Akira et al., 2006; Karin et al., 2006; Kawai and Akira, 2006).

The innate response is invariable and works by using a transmitted germ-line coded pool of specific receptors (Janeway, 1989; Medzhitov, 2001). These receptors are expressed on NK cells, γδ-T-cells and CD5+ B-cells that cover a broad spectrum of different antigens (Chalifour et al., 2004; Dono et al., 2004). They belong to a recently discovered family of non-clonally expressed pattern recognition receptors that show homology with the *Drosophila* Toll protein and the human interleukin-1 receptor family (Medzhitov and Janeway, 2000). These Toll-like receptors (TLRs) don't recognize specific single structures, but specific patterns, termed pathogen-associated molecular patterns. These specific patterns are conservative structures like carbohydrates on glycoproteins and glycolipids and repetitive structures (e.g. LPS) (Janeway, 1989; Medzhitov and Janeway, 2000; Janeway and Medzhitov, 2002; Pasare and Medzhitov, 2005) and they are expressed independently from mutational events (Janeway, 1989). This recognition system guarantees that the immune response need not follow all mutational changes, but can focus on the detection of structures that are most likely involved in primary cell stability and cell preservation

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mechanisms.

In particular, natural IgM antibodies play an important role in primary defense mechanisms (Casali and Notkins, 1989; Vollmers et al., 1989; Bohn, 1999; Boes, 2000; Brändlein et al., 2003b). They are known to be involved in early recognition and elimination of external invaders like bacteria and viruses, cellular waste and modified self (Janeway et al., 1989; Ben-Aissa-Fennira et al., 1998; Boes et al., 1998; Ochsenbein et al., 1999; Ulvestad et al., 2001; Milner et al., 2005; Peng et al., 2005).

By now, sufficient evidence has been collected to show that it is the innate immunity and the natural IgM antibodies which seem to be involved in the immune surveillance mechanisms against precancerous and cancerous cells (Figs. 1, 2) (Vollmers et al., 1995; Hensel et al., 1999b, 2001b; Glennie and van de Winkel 2003; Brändlein et al., 2002, 2003a, b, 2004b; Brändlein and Vollmers 2004; Vollmers and Brändlein 2002, 2005a,b).

The germ-line code

The innate immune system requires enough genetic variability to cover a very broad spectrum of foreign antigens which ensures that we are not overcome by the multitude of infections or malignant cells that challenge the average human body over the period of a standard lifetime. Natural IgM antibodies are not affinity maturated and they are coded by specific germ-line gene families (Baumgarth et al., 2005). Over 80% of the tumour-reactive monoclonal antibodies were expressed by VH genes of the VH3 gene family. Within this family especially the germ-line genes DP47 and DP49 were overrepresented (Brändlein et al., 2003b). The expression of these germ-line genes was found to be significantly higher than described earlier (Brezinschek et al., 1995). This preferential utilization of the VH3 gene family by tumour-specific antibodies is close to what is known about the specific immune reaction against pathogens. In the humoral defence mechanisms against infectious particles like bacteria and viruses some genes are also found to be involved more frequently than would be expected by random selection (Adderson et al., 1991; Silverman and Lucas, 1991; Huang et al., 1992) (see Table 1).

Genetic restriction is not limited to heavy chain genes, it is also found within VL genes. More than 90% of the tumour-reactive monoclonal antibodies isolated from cancer patients or healthy donors utilized λ-light chain genes (Hensel et al., 1999a; Brändlein et al., 2003b). These results are significantly different from those of the usage of λ-light chains in the unstimulated B cell repertoire, where only 40% of the light chains belong to the λ-isotype. Again, a couple of VL gene families are overrepresented (Table 1). The degree of identity of the VL regions of tumour-reactive monoclonal antibodies to their most homologous VL germ-line genes ranges between 97,2 and 100%, which is similar to the identity of the VH genes to the homologous germ-line genes (Brändlein et al., 2003b). The high homology of the VH- and VL-regions to the germ-line genes indicate that the antibodies isolated either from cancer patients or from healthy individuals are not affinity-maturated by somatic mutation due to antigen contact (Brändlein et al., 2003b).

Taken together, the antibody repertoire is genetically limited and no or only few mutations expand this set. However, a certain acquired genetic variability of the innate immunoglobulin receptors is achieved by combinatorial association of germ-line immunoglobulin genes. Further deletions, additions and mistakes in recombination events guarantee a genetic and receptor variability which is sufficient to cover a broad spectrum of antigens on pathogenic organisms and gives a sufficient protection without additional mutational adaptation (Schatz et al., 1992; Lewis, 1994; Constantinescu and Schissel, 1997; Gellert, 1997; Papavasiliouet al., 1997; Cedar and Bergman, 1999; Rothenberg, 2000).

The low affinity

Major changes on malignant cells are post-transcriptional modifications of carbohydrate residues on cell surface glycolipids and glycoproteins (Lloyd and Old, 1989; Turner, 1992; Egea et al., 1993; Hanisch et al., 1996; Baldus et al., 2004; Cobb and Kasper, 2005). These differences in comparison to the normal counterparts seem to be important for malignant growth, movement and adhesion (Vlad and Finn, 2004; Kannagi et al., 2004; Dube and Bertozzi, 2005).

Cancer-associated changes in glycosylation include both the under- and overexpression of naturally occurring glycans as well as neoexpression of glycans (Dwek and Brooks, 2004). These "tumour-associated carbohydrate antigens" are prominent targets of immune

Table 1. Gene families of tumour-specific IgM.

LYMPHOCYTE ORIGIN	VH FAMILY	VH SUBGROUP	VL FAMILY	VL SUBGROUP
Stomach cancer patient	VH3, VH4	DP-42, DP-47, DP-49	λI, λIII	IgLV3-1, -21 IgLV1-40, -51
Lung cancer patient	VH4	DP-65	-	-
Colon cancer patient	VH3, VH5	DP-49, DP-73	λI, λII	IgLV2-14 IgLV3-25
Pancreas cancer patient	VH3	DP-47	λIII, λV	IgLV3-10 IgLV5-45
Healthy donor	VH3, VH4	DP-47, DP-49, DP-77	λI, λIII	IgLV3-10 IgLV1-40, -47

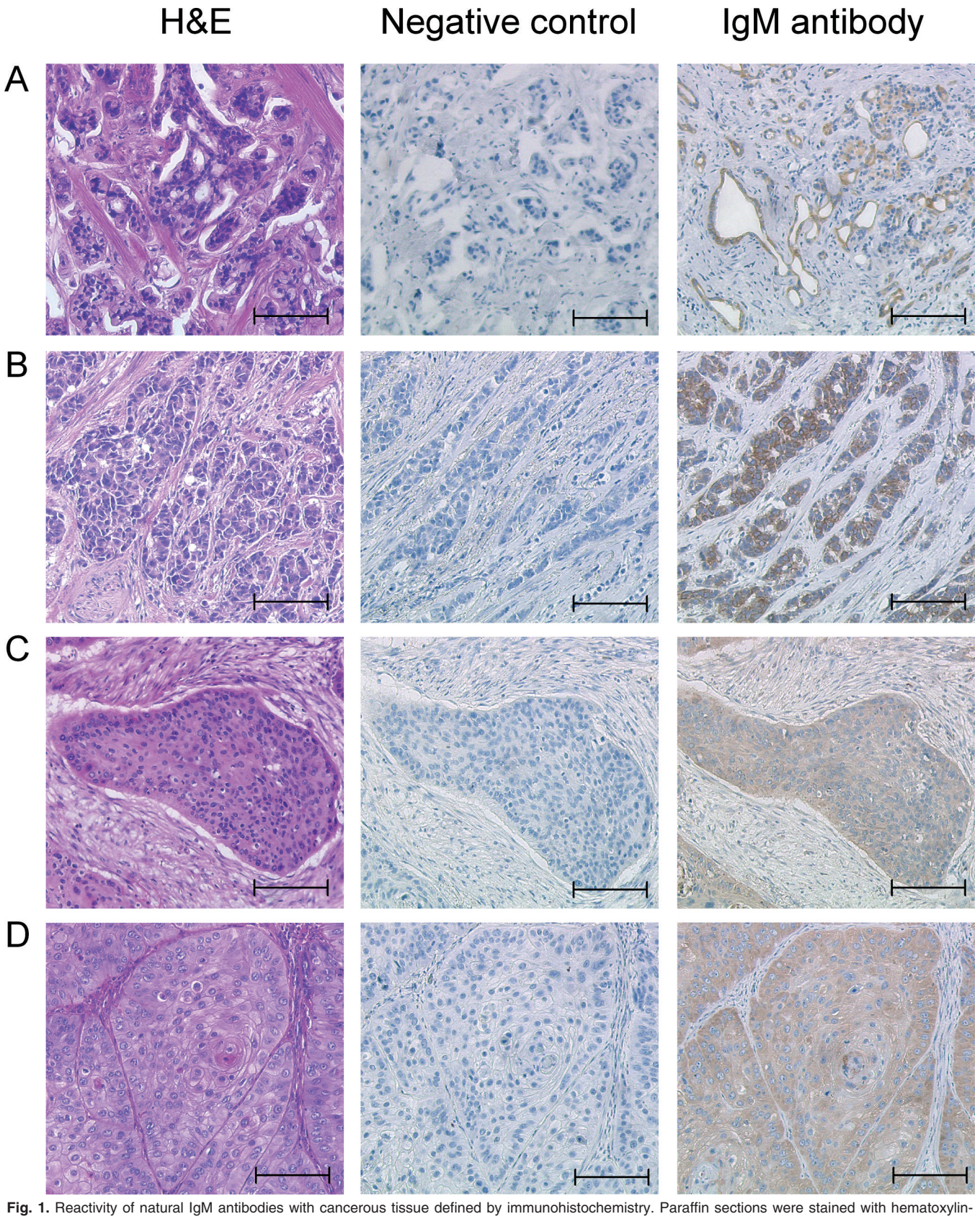

Fig. 1. Reactivity of natural IgM antibodies with cancerous tissue defined by immunohistochemistry. Paraffin sections were stained with hematoxylin-eosin, isotype matched control antibody as a negative control and several IgM antibodies isolated from tumour patients or healthy donors. **A.** adenocarcinoma of the pancreas. **B.** invasive lobular carcinoma of the breast. **C.** squamous cell carcinoma of the esophagus. **D.** squamous cell carcinoma of the cervix. Bars: 100 µm.

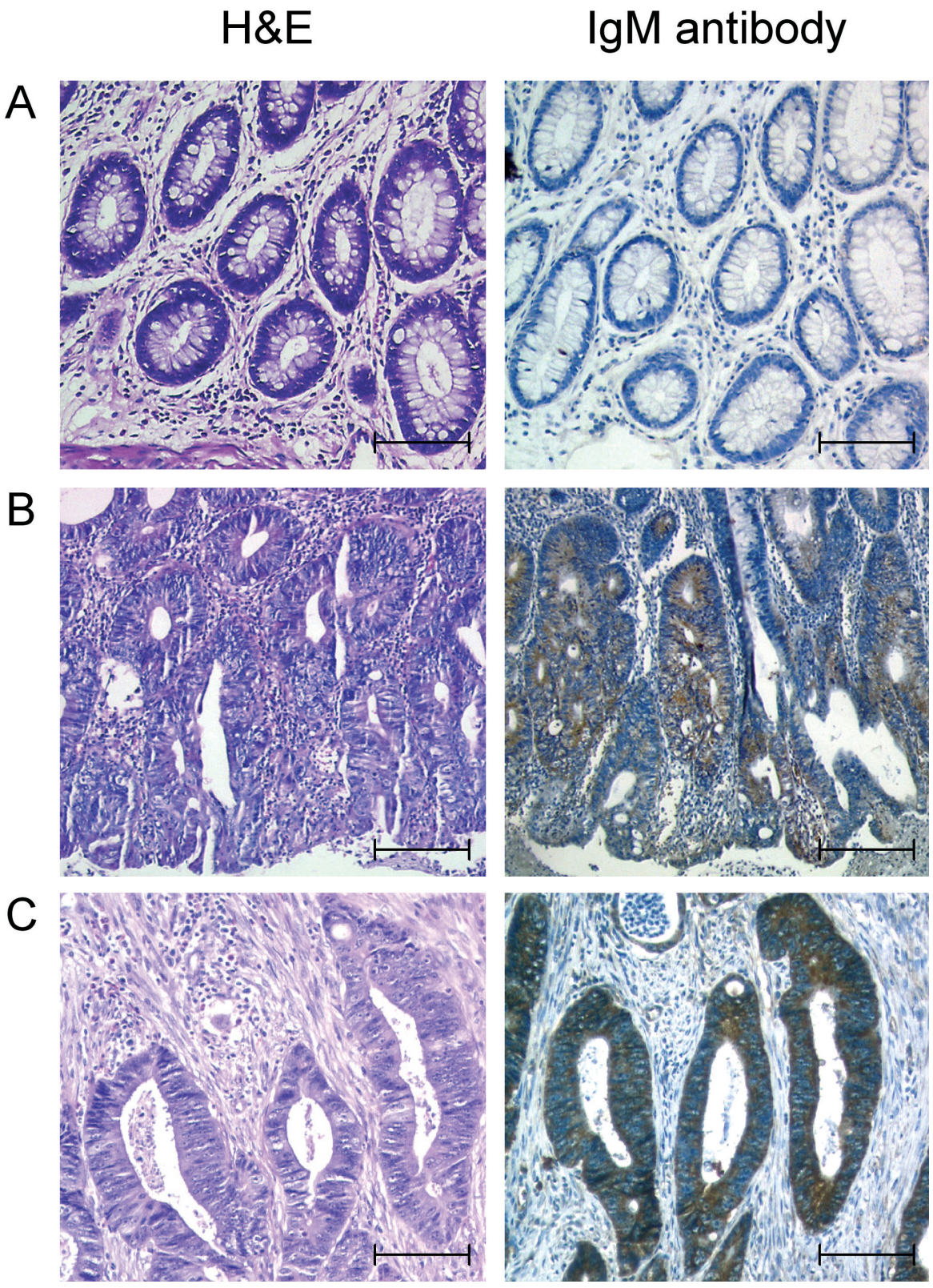

Fig. 2. Reactivity of natural IgM antibody with precancerous and cancerous colon mucosa defined by immunohistochemistry. Paraffin sections were stained with hematoxylin-eosin, isotype matched control antibody as a negative control and IgM antibody PAM-1 which was isolated from a patient with stomach carcinoma. **A.** healthy colon mucosa. **B.** ulcerative colitis-related dysplasia. **C.** adenocarcinoma of the colon. Bars: 100 µm.

surveillance and natural IgM antibodies (Hensel et al., 1999a, 2001a; Brändlein et al., 2003b).

One example is the cell surface molecule DAF (decay acceleration factor, also named CD55) which prevents cell lysis by autologous complement (Davis et al., 1988; Kuraya and Fujita, 1998). It is present in different isoforms on all cells of an organism (Caras et al., 1987). The natural IgM antibody SC-1 binds to a specific modified isoform of DAF-B (decay acceleration factor, CD55), exclusively expressed on the membrane of stomach carcinoma cells (Vollmers et al., 1989; Hensel et al., 1999b, 2001b). This modified molecule (CD55/SC-1) is co-expressed with the wild-type of CD55 on the surface of stomach cancer cells (Hensel et al., 2001b). The antibody SC-1 binds to a tumour-specific carbohydrate epitope of CD55 and induces apoptosis of stomach cancer cells both in vitro (Vollmers et al., 1997; Hensel et al., 1999a, 2001b) and in experimental *in vivo* systems (Vollmers et al., 1995; 1998a,b).

Growth factor receptors like EGFR or FGFR are often over-expressed on malignant cells, because faster and aggressive growth requires a higher uptake and turnover of energy.

The blocking of these receptors leads to starvation and cell death. The human germ-line coded monoclonal

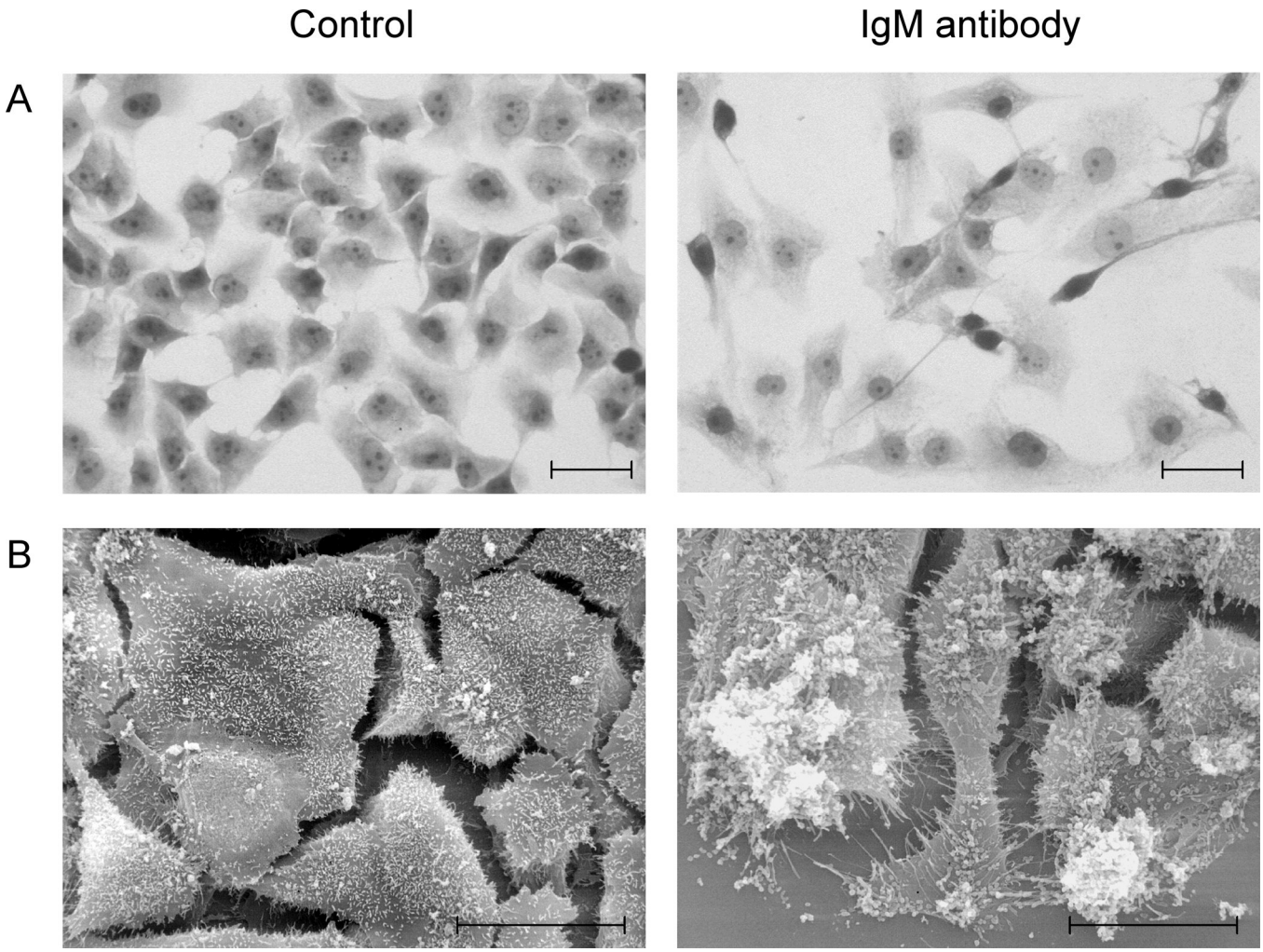

Fig. 3. Natural IgM antibody induced tumour cell apoptosis *in vitro*. **A.** IgM antibody induced apoptosis *in vitro*: Pancreas carcinoma cell line BXPC-3 was incubated with IgM antibody and isotype control for 48 h. IgM antibody-induced changes of tumour cell morphology are visible after 48h. The cells treated with IgM antibody are more spindle-shaped and flat, more polarized with more pronounced cytoplasmic elongations. Furthermore, a loss of cell-cell contacts and adhesion was observed. In contrast, untreated tumour cells grow in homogenous mono-layers. Bars: 20 µm. **B.** Scanning electron microscopy of IgM antibody induced apoptosis. Stomach carcinoma cell line 23132/87 was incubated with a natural IgM antibody isolated from a healthy individual at a concentration of 10µg/ml for 24h. As a control, cells were treated with unrelated human control IgM. Samples were processed for scanning electron microscopy and analysed by ZEISS DSM 962. On the shrunken tumour cells, packages of membrane vesicles, apoptotic bodies, are clustered and stress fibres are visible. Bars: 50 µm.

IgM antibody PAM-1 binds to a membrane receptor, which was found to be a 130 kDa integral membrane glycoprotein (Vollmers et al., 1994; Hensel et al., 2001a) homologous to CFR-1 (cysteine-rich fibroblast growth factor receptor) (Burrus et al., 1992). This post-transcriptionally modified CFR-1/PAM-1 receptor is expressed on almost all epithelial cancers of every type and origin and on precursor stages, but not on healthy tissue (Hensel et al., 2001a; Brändlein et al., 2003a, 2004c). Binding of antibody PAM-1 induces apoptotic events *in vitro* and *in vivo* (Brändlein et al, 2004b).

The carbohydrate epitopes of the investigated IgM antibodies are relatively stably expressed on a variety of tumours and additionally on precursor stages (Figs. 1, 2). Furthermore, they are found on primary cancers as well as on derived lymph node and liver-metastases.

Carbohydrates are rediscovered as immune epitopes and immunity is no longer solely a matter of peptide recognition and presentation (Carter et al., 2004). Unlike classical peptide-chain-based epitopes, glyco-epitopes can share significant structural homologies beyond the limits of protein families. These glyco-epitopes are thus prone to cross-react extensively and are therefore preferential targets for natural IgM antibodies. The low affinity of natural IgM antibodies enables these molecules to detect specific patterns of carbo-epitopes which are present on different molecules.

In addition, a high affinity is not a prerequisite for functional activity. All investigated natural IgM antibodies show a strong apoptotic activity on cancer cells (Brändlein et al., 2003b; Vollmers and Brändlein 2005a). It is likely that the lack of affinity might be compensated by the pentameric avidity (Fig. 3).

The oligo-reactivity

Based on Burnet's clonal selection theory (Burnet, 1957) a given lymphocyte is genetically programmed to produce a single mono-specific antibody (Tonegawa, 1988). About 20 years ago, the first human monoclonal antibodies against malignancies established by using hybridoma technology, caused some surprise. Many of the tumour-reactive monoclonal antibodies were not mono-specific but instead cross-reacted with a variety of different and totally unrelated antigens (Dighiero et al., 1983; Haspel et al., 1983; Ternynck and Avrameas, 1986; Casali and Notkins, 1989). Today we know that these antibodies in fact represent the anti-tumour activity of the host and that their cross-reactivity is a prerequisite for survival, recognizing and removing non-self structures on tumour-transformed and deranged or functionally impaired self-structures, invading microbes and virus-infected cells (Ochsenbein et al., 1999; Boes, 2000).

Natural antibodies are rather “oligo-specific” than “poly-specific” or even “non-specific” (Vollmers and Brändlein, 2002), as they are often referred to in the literature. As mentioned above, these IgM antibodies act as a kind of pattern recognition receptors and most of them show this overlapping activity, which is hardly surprising considering the fact that a limited inherited set of receptors has to cover a relatively broad spectrum of “non-self”. The specific oligo-reativity of individual natural antibodies is important because it provides pre-existing antibody reactivity enabling animals to rapidly recognize and eliminate non-self structures which have not been encountered previously.

Originally, it was thought that oligo-reactive antibodies were exclusively auto-antibodies, but soon it became apparent that this was not the case because these antibodies bound just as well to many foreign antigens and were furthermore found in all immuno-competent individuals (Prabhakar et al., 1984). Most oligo-reactive antibodies belong to the IgM class, but some are IgG and IgA and can bind to proteins, carbohydrates, lipids and nucleic acids. From an evolutionary point of view, oligo-reactivity is a highly conserved feature of the immune system and oligo-reactive antibodies are found in species as distantly related as humans and sharks (Marchalonis et al., 2001). Today we know that cross-reactivity of natural IgM antibodies is a typical and necessary feature associated with their function as street-sweepers and first line defenders. Being faced with a huge amount of “non-self” particles and structures and being equipped with only a limited set of receptor genes, nature has developed an economic recognition system directed against conservative structures which are shared by different carriers.

A very sophisticated and so far unknown mechanism of antibody-crossover and induction of tumour cell apoptosis is shown by the IgM antibody SAM-6. This tumour-specific antibody induces accumulation of neutral and polar lipids in tumour cells, but not in normal cells (Fig. 4) (Pohle et al., 2004). The accumulation of lipids is responsible for the tumour cell apoptosis which can be observed as a formation of apoptotic bodies via scanning electron microscopy analysis. Neutral lipids like triglycerides and cholesterol are essential for each cell. Binding and uptake of lipids, e.g. LDL, is regulated strictly. A balanced lipid metabolism is crucial for all cells. Disturbance of this homeostasis by non-physiological intracellular accumulation of fatty acids can result in apoptosis. This has been proven in animal studies and has been correlated to some human diseases, such as lipotoxic cardiomyopathy. Some metabolic mechanisms of lipo-apoptosis have been described and some causes are discussed, but reagents which directly induce these syndromes have not been identified so far. In normal states a feedback mechanism guarantees an inhibition of overtake. In certain cardiac diseases however, an over-accumulation of lipids by heart muscle cells leads to cell death by apoptosis (Narula et al., 1999; Kang and Izumo, 2000). The cell is no longer able to manage the lipids and dies, with fatal consequences for the organism.

The unique functional activity of antibody SAM-6 to overfeed cancer cells with lipoproteins shows nature´s skills in eliminating transformed cells and is also a good

example of innate immune receptor economy.

The pentameric structure

A relevant percentage of the blood-circulating immunoglobulins are pentameric IgMs. Additionally they can be found in the gastrointestinal tract, lymphatic vessels, mucosal tissues, bone marrow etc. However, one of the most prominent arguments against the use of native IgM molecules for cancer therapy is that a pentameric IgM molecule with a mass of approximately 1 Mill kDa cannot pass the blood-tissue endothelial barrier and does not penetrate surrounding tissue (Jain and Baxter, 1988; Adams et al., 2001). This dogma is mainly based on observations of experimental animal systems where mice or other animals were inoculated with human tumour cells, mostly via the subcutaneous route, and the subsequent diffusion and penetration

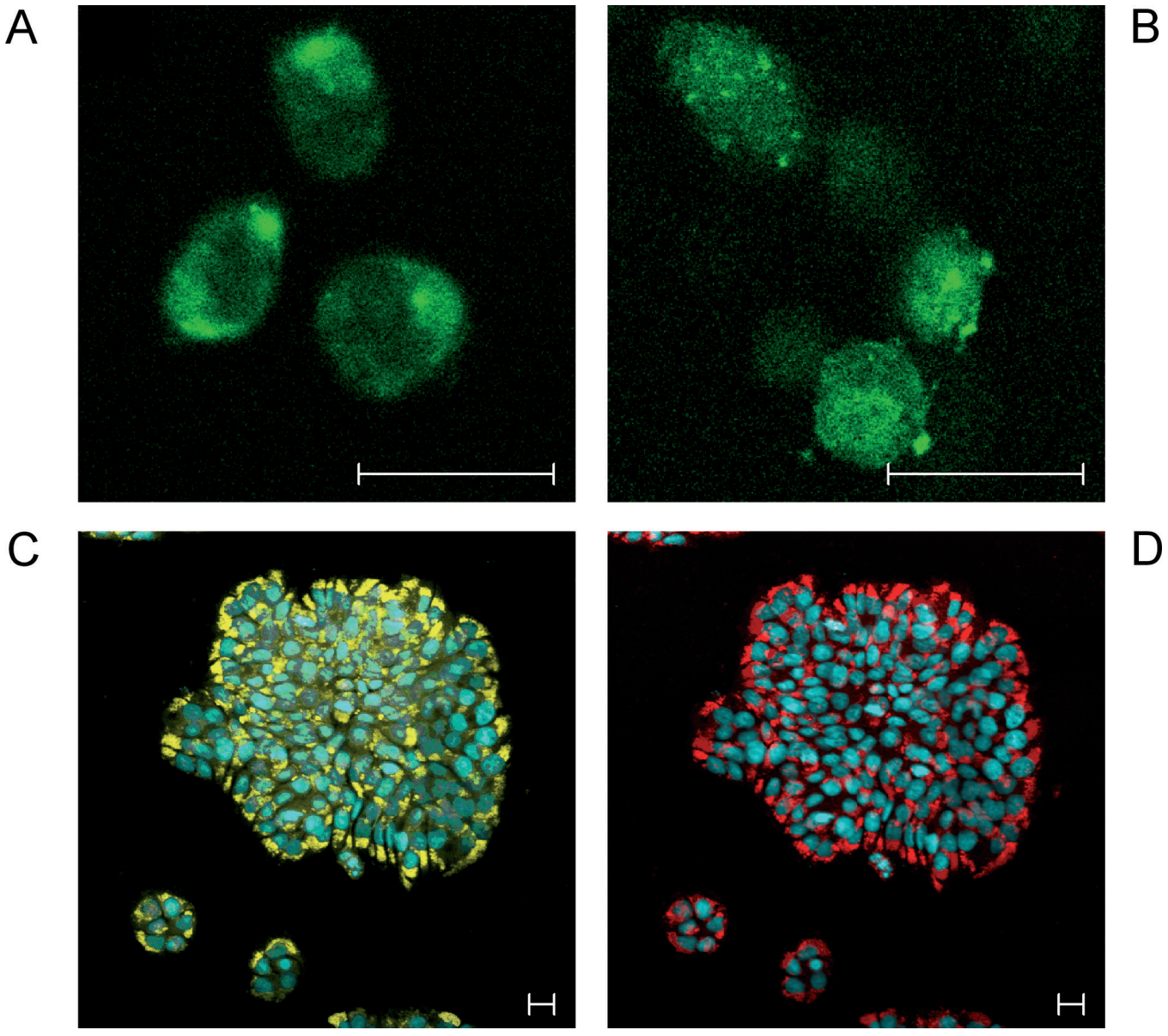

Fig. 4. Endocytosis of natural IgM antibody SAM-6 and lipid accumulation. **A, B.** Immunofluorescence of SAM-6 endocytosis on pancreas cancer cell line BXPC-3. The pancreas cancer cells were incubated with IgM antibody SAM-6 for the indicated period of time and processed for immunoflourescence microscopy. 60 min: capping **(A)**; 120 min: endocytosis **(B)**. **C, D.** Nile red staining of neutral and polar lipids in SAM-6 induced apoptosis on tumour cells. Gastric carcinoma cell line 23132/87 was incubated with IgM antibody SAM-6 or unrelated human control IgM antibody for 48h. Neutral lipids are stained yellow (488 nm) **(C)**, polar lipids are stained dark red (543 nm) **(D)** and cell nuclei appear blue (350 nm). Bars: 100 µm.

patterns of radiolabeled antibodies was measured. Generally, only small antibody fragments led to a high accumulation, whereas larger molecules predominantly remained in the bloodstream (Colcher et al., 1990; Milenic et al., 1991; Adams et al., 1993; Todorovska et al., 2001). In comparison with small molecules, intact molecules naturally show a slower penetration over time, but even intact IgM antibodies reach implanted tumours in mice and also primary tumours and metastases in patients after i.v. or i.p. administration (Vollmers et al., 1998a,b).

In general, experimental tumours in mice cannot be

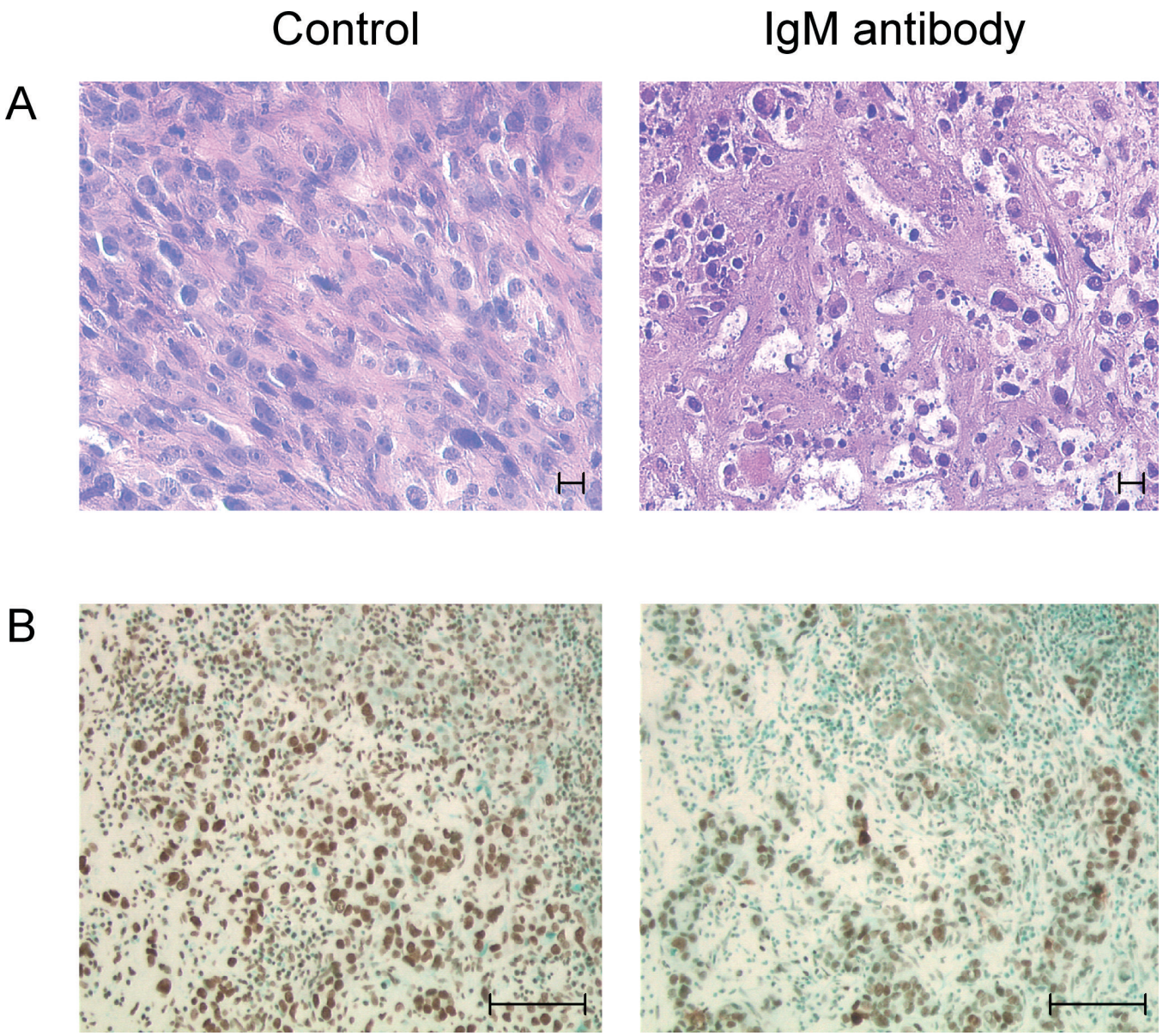

Fig. 5. *In vivo* activity of human IgM antibodies. **A.** Morphological analysis of *in vivo* activity of antibody SAM-6. Immunohistochemical staining with H&E was prepared on paraffin sections of human pancreas carcinoma grown s.c. in scid-mice. Mice were treated i.p. with antibody SAM-6 or human unrelated IgM control. Mice treated with the pentameric IgM antibody SAM-6 show, besides reduced tumour growth, morphological changes like regression and apoptotic cells. **B.** SC-1 induced apoptosis after carbohydrate binding on stomach carcinoma cells in liver metastasis Analysis of antibody SC-1 induced apoptotic activity *in vivo*. Apoptotic stomach carcinoma cells in a liver metastasis of a patient after treatment with the antibody SC-1. The patient received a single dose of antibody SC-1 before surgery and the liver metastasis was investigated for SC-1 induced apoptosis using the Klenow FragEL DNA fragmentation Kit (Oncogene, Boston, USA). In the positive control all cell nuclei are stained because of treatment with an endonuclease. In the left picture only the nuclei of apoptotic stomach tumour cells are stained and normal non-malignant liver cells are not affected. Bars: 100 µm.

compared with human neoplasm and almost all results obtained, concerning human antibody activity, specificity and transport, are of only limited value. In addition, the majority of tumour targeting experiments have been performed so far with antibodies directed against structures that are not tumour-specific at all, such as CEA, TAG-72, EPG-2, 17-1A etc. (Todorovska et al., 2001). One must therefore consider the possibility that the experimental data, which discredits larger molecules, are the result of poor specificity and passive diffusion of the antibodies and not of specific targeting.

However, it could be demonstrated that pentameric IgMs, when injected i.p., reach subcutaneously transplanted tumours on the back of the animals. Size reduction, clearly based on antibody-induced apoptotic effects, could be demonstrated for these tumours (Fig. 5a). This shows that pentameric molecules are able to leave the intraperitoneal cavity, enter the circulation and reach the implanted tumour. On their way, the antibodies have to pass several endothelial barriers of lymphatic and blood vessels before they reach their targets (Denekamp, 1984; Dvorak, 1990; Mostov, 1994; Michel, 1996; Jain, 2001).

The best argument against the "pentameric IgM antibodies do not cross the endothelial barrier" dogma, is the example of human monoclonal IgM antibody SC-1, which was used in a clinical trial in stomach carcinoma patients. In this study patients diagnosed with primary stomach tumours received a single dose of SC-1 antibody intravenously. Gastrectomy was performed two days after the injection. Significant morphological and apoptotic changes could be observed in primary tumours and metastases as well (Fig. 5b) (Vollmers et al., 1998a,b). This proves that pentameric IgM molecules are able to leave the circulation, cross the endothelia and matrices to reach the interstitium and the tumour, and to specifically kill tumour cells *in vivo*.

Taken together, it is likely that IgM penetration is slower than that of monomeric or fragmented immunoglobulins. Nevertheless, IgMs reach their targets, no matter where they are injected and which barriers they have to pass in animals or humans. In addition, a high accumulation and fast tumour-penetration of antibodies is only required for certain treatments, e.g. if antibodies are used as carriers. If they are equipped with a biological activity like induction of apoptosis, slower penetration and accumulation could be an advantage.

The "no need" for memory

The innate immunity triggers the secondary immune response to induce the adaptive immunity and to create a memory system (Ochsenbein and Zinkernagel, 2000; Iwasaki and Medzhitov, 2004; Pasare and Medzhitov, 2005). This has been demonstrated for infectious diseases on cellular and humoral level (Hoebe et al., 2004; Raulet, 2004; Degli-Esposti and Smith, 2005; Hamerman et al., 2005; deVisser et al., 2006). For cancer, however, so far no tumour-specific T or B memory cells have been detected and no affinity-maturated IgG antibodies have been isolated. In opposite, nearly all tumour-specific antibodies are of IgM type and belong to the innate immunity (Brändlein, 2003b). Although natural IgM antibodies act as first line defenders against all kind of invaders and remove as "street sweepers" all kind of o cellular waste, dangerous modified cells and molecules, there seems to be no switch to maturation. The induction of adaptive immunity is T-cell dependent, what means that T-cells recognize and present foreign molecules in context with self (Langman and Cohn, 2002). However, only proteins are processed by the adaptive immune machinery and are able to create a memory and maturation. Other structures, like carbohydrates, are antigenic and immunogenic, but cannot be processed and presented as foreign motifs to other immune competent cells.

However, many of the tumour-specific changes on cancer cells have been proven to be alterations in glyco-structures (Kobata and Amano, 2005) and these structures are detected by the innate immune receptors in a T-cell independent way (Janeway, 1989; Hensel et al., 1999b, 2001b; Brändlein et al., 2003b; Karin et al., 2006). In addition tumour cells don't hide intra-cellularly somewhere in the body or re-infect an organism. Furthermore, most of the transformed cells are detected at a very early stage, and manifest cancers are the exceptions. Based on this, it is not surprising that a switch and an affinity maturation is not initiated. And there seems to be no need for an anti-cancer memory, otherwise, nature would have created one.

Final remarks

Natural immunity is the most critical and most important force in the detection and elimination of transformed cells and natural antibodies, with their ideal characteristics, are natures´ best weapons to fight cancer. Natural IgM antibodies, treated by most immunologists for a long time as parias, are back on stage. After about 25 years of xeno-immunizations, recombinations and all kinds of antibody improvements, we believe it is time now to accept that the best therapeutic antibodies already exist in humans and are ready to be used.

References

Adams G.P., McCartney J.E., Tai M.S., Oppermann H., Huston J.S., Stafford 3rd W.F., Bookman M.A., Fand I., Houston L.L. and Weiner L.M. (1993). Highly specific in vivo tumor targeting by monovalent and divalent forms of 741F8 anti-c-erbB-2 single-chain Fv. Cancer Res. 53, 4026-4034.

Adams G.P., Schier R., McCall A.M., Simmons H.H., Horak E.M., Alpaugh R.K., Marks J.D. and Weiner L.M. (2001). High affinity restricts the localization and tumor penetration of single-chain fv antibody molecules. Cancer Res. 61, 4750-4755.

Akira S., Uematsu S. and Takeuchi O. (2006). Pathogen recognition and innate immunity. Cell 124, 783-801.

Adderson E.E., Shackelford P.G., Quinn A. and Carroll W.L. (1991). Restricted Ig H chain V gene usage in the human antibody response to Haemophilus influenzae type b capsular polysaccharide. J. Immunol. 147, 1667-1674.

Baldus S.E., Engelmann K. and Hanisch F.G. (2004). MUC1 and the MUCs. a family of human mucins. Crit. Rev. Clin. Lab. Sci. 41, 189-231.

Baumgarth N., Tung J.W. and Herzenberg L.A. (2005). Inherent specificities in natural antibodies: a key to immune defense against pathogen invasion. Springer Semin. Immunopathol. 26, 347-362.

Ben-Aissa-Fennira F., Ben Ammar-El Gaaied A., Bouguerra A. and Dellagi K. (1998). IgM antibodies to P1 cytoadhesin of Mycoplasma pneumoniae are part of the natural antibody repertoire expressed early in life. Immunol. Lett. 63, 59-62.

Boes M. (2000). Role of natural and immune IgM antibodies in immune responses. Mol. Immunol. 37, 1141-1149.

Boes M., Prodeus A.P., Schmidt T., Carroll M.C. and Chen J. (1998). A critical role of natural immunoglobulin M in immediate defense against systemic bacterial infection. J. Exp. Med. 188, 2381-2386.

Bohn J. (1999). Are natural antibodies involved in tumour defence? Immunol. Lett. 69, 317-320.

Brändlein S. and Vollmers H.P. (2004). Natural IgM antibodies, the ignored weapons in tumour immunity. Histol. Histopathol. 19, 897-905.

Brändlein S., Lorenz J., Ruoff N., Hensel F., Beyer I., Müller J., Neukam K., Illert B., Eck M., Müller-Hermelink H.K. and Vollmers H.P. (2002). Human monoclonal IgM antibodies with apoptotic activity isolated from cancer patients. Hum. Antibodies 11, 107-119.

Brändlein S., Beyer I., Eck M., Bernhardt W., Hensel F., Müller-Hermelink H.K. and Vollmers H.P. (2003a). CFR-1, a new marker for precancerous epithelial lesions defined by the human monoclonal antibody PAM-1. Cancer Res. 63, 2052-2061.

Brändlein S., Pohle T., Ruoff N., Wozniak E., Müller-Hermelink H.K. and Vollmers H.P. (2003b). Natural IgM antibodies and immunosurveillance mechanisms against epithelial cancer cells in humans. Cancer Res. 63, 7995-8005.

Brändlein S., Pohle T., Vollmers C., Wozniak E., Ruoff N., Müller-Hermelink H.K. and Vollmers H.P. (2004a). CFR-1 receptor as target for tumor-specific apoptosis induced by the natural human monoclonal antibody PAM-1. Oncol. Rep. 11, 777-784.

Brändlein S., Eck M., Strobel P., Wozniak E., Muller-Hermelink H.K., Hensel F. and Vollmers H.P. (2004b). PAM-1, a natural human IgM antibody as new tool for detection of breast and prostate precursors. Hum. Antibodies 13, 97-104.

Brezinschek H.P., Brezinschek R.I. and Lipsky P.E. (1995). Analysis of the heavy chain repertoire of human peripheral B cells using single-cell polymerase chain reaction. J. Immunol. 155, 190-202.

Burnet F.M. (1957). A modification of Jerne's theory of antibody production using the concept of clonal selection. Austr. J. Sci. 20, 67–68.

Burnet F.M. (1964). Immunological factors in the process of carcinogenesis. B. Med. Bull. 20, 154-158.

Burnet F.M. (1970). The concept of immunological surveillance. Prog. Exp. Tumor Res. 13, 1-27.

Burrus J.W., Zuber M.E., Lueddecke B.A. and Olwin B.B. (1992). Identification of a cysteine-rich receptor for fibroblast growth factors. Mol. Cell Biol. 12, 5600-5609.

Caras I.W., Davitz M.A., Rhee L., Weddell G., Martin D.W.J. and Nussenzweig V. (1987). Cloning of decay-accelerating factor suggests novel use of splicing to generate two proteins. Nature 325, 545-549.

Carter P., Smith L. and Ryan M. (2004). Identification and validation of cell surface antigens for antibody targeting in oncology. Endocr. Relat. Cancer 11, 659-687.

Casali P. and Notkins A.L. (1989). CD5+ B lymphocytes, polyreactive antibodies and the human B-cell repertoire. Immunol. Today 10, 364-368.

Cedar H. and Bergman Y. (1999). Developmental regulation of immune system gene rearrangement. Curr. Opin. Immunol. 11, 64-69.

Chalifour A., Jeannin P., Gauchat J.F., Blaecke A., Malissard M., N'Guyen T., Thieblemont N. and Delneste Y. (2004). Direct bacterial protein PAMP recognition by human NK cells involves TLRs and triggers alpha-defensin production. Blood 104, 1778-1783.

Cobb B.A. and Kasper D.L. (2005). Coming of age. carbohydrates and immunity. Eur. J. Immunol. 35, 352-356.

Colcher D., Bird R., Roselli M., Hardman K.D., Johnson S., Pope S., Dodd S.W., Pantoliano M.W., Milenic D.E. and Schlom J. (1990). In vivo tumor targeting of a recombinant single-chain antigen-binding protein. J. Natl. Cancer Inst. 82, 1191-1197.

Constantinescu A. and Schlissel M.S. (1997). Changes in locus-specific V(D)J recombinase activity induced by immunoglobulin gene products during B cell development. J. Exp. Med. 185, 609-620.

Davis L.S., Patel S.S., Atkinson J.P. and Lipsky P.E. (1988). Decay-accelerating factor functions as a signal transducing molecule for human T cells. J. Immunol. 141, 2246-2252.

Degli-Esposti M.A. and Smyth M.J. (2005). Close encounters of different kinds: dendritic cells and NK cells take centre stage. Nat. Rev. Immunol. 5, 112-124.

Denekamp J. (1984). Vascular endothelium as the vulnerable element in tumours. Acta Radiol. Oncol. 23, 217-225.

De Visser K.E., Eichten A. and Coussens L.M. (2006). Paradoxical roles of the immune system during cancer development. Nature Rev. Cancer 6, 24-37.

Dighiero G., Lymberi P., Mazie J.C., Rouyre S., Butler-Browne G.S., Whalen R.G. and Avrameas S. (1983). Murine hybridomas secreting natural monoclonal antibodies reacting with self antigens. J. Immunol. 131, 2267–2272.

Dono M., Cerruti G. and Zupo S. (2004). The CD5+ B-cell. Int. J. Biochem. Cell Biol. 36, 2105-2111.

Dube D.H. and Bertozzi C.R. (2005). Glycans in cancer and inflammation--potential for therapeutics and diagnostics. Nat. Rev. Drug Discov. 4, 477-488.

Dunn G.P., Old L.J. and Schreiber R.D. (2004). The three Es of cancer immunoediting. Annu. Rev. Immunol. 22, 329-360.

Dvorak H.F. (1990). Leaky tumor vessels. consequences for tumor stroma generation and for solid tumor therapy. Prog. Clin. Biol. Res. 354A, 317-330.

Dwek M.V. and Brooks S.A. (2004). Harnessing changes in cellular glycosylation in new cancer treatment strategies. Curr. Cancer Drug Targets 4, 425-442.

Egea G., Franci C., Gambus G., Lesuffleur T., Zweibaum A. and Real F.X. (1993). cis-Golgi resident proteins and O-glycans are abnormally compartmentalized in the RER of colon cancer cells. J. Cell Sci. 105, 819-830.

Ehrlich P. 73 Verh. Ges. Dtsch. Naturforsch. Aerzte 1 (1902) 250–275 (Translations. Ehrlich, P. in Collected Studies on Immunity, p. 388,Wiley, New York, 1906.)

Gellert M. (1997). Recent advances in understanding V(D)J

recombination. Adv. Immunol. 64, 39-64.

Glennie M.J. and van de Winkel J.G. (2003). Renaissance of cancer therapeutic antibodies. Drug Discov. Today 1, 503-510.

Greenberg S. and Grinstein S. (2000). Phagocytosis and innate immunity. Curr. Opin. Immunol. 14, 136-145.

Hamerman J.A., Ogasawara K. and Lanier L.L. (2005). NK cells in innate immunity. Curr. Opin. Immunol. 17, 29-35.

Hanisch F.G., Stadie T.R., Deutzmann F. and Katalinic J.P. (1996). MUC1 glycoforms in breast cancer-cell line T47D as a model for carcinoma-associated alterations of 0-glycosylation. Eur. J. Biochem. 236, 318-327.

Haspel M.V., Onodera T., Prabhakar B.S., McClintock P.R., Essani K., Ray U.R., Yagihashi S. and. Notkins A.L. (1983). Multiple organ-reactive monoclonal autoantibodies. Nature 304, 73-76.

Hensel F., Knorr C., Hermann R., Krenn V., Muller-Hermelink H.K. and Vollmers H.P. (1999a). Mitogenic autoantibodies in Helicobacter pylori-associated stomach cancerogenesis. Int. J. Cancer 81, 229-235.

Hensel F., Hermann R., Schubert C., Abé N., Schmidt K., Franke A., Shevchenko A., Mann M., Müller-Hermelink H.K. and Vollmers H.P. (1999b). Characterisation of glycosylphosphatidylinositol-linked molecule CD55/ Decay-accelerating factor as the receptor for antibody SC-1-induced apoptosis. Cancer Res. 59, 5299-5306.

Hensel F., Brändlein S., Eck M., Schmidt K., Krenn V., Kloetzer A., Bachi A., Mann M., Muller-Hermelink H.K. and Vollmers H.P. (2001a). A novel proliferation-associated variant of CFR-1 defined by a human monoclonal antibody. Lab. Invest. 81, 1097-1108.

Hensel F., Hermann R., Brandlein S., Krenn V., Schmausser B., Geis S., Muller-Hermelink H.K. and Vollmers H.P. (2001b). Regulation of the New Coexpressed CD55 (Decay-Accelerating Factor) Receptor on Stomach Carcinoma Cells Involved in Antibody SC-1-Induced Apoptosis. Lab. Invest. 81, 1553-1563.

Hoebe K., Janssen E. and Beutler B. (2004). The interface between innate and adaptive immunity. Nat. Immunol. 5, 971-974.

Huang D.F., Olee T., Masuho Y., Matsumoto Y., Carson D.A. and Chen P.P. (1992). Sequence analyses of three immunoglobulin G anti-virus antibodies reveal their utilization of autoantibody-related immunoglobulin Vh genes, but not V lambda genes. J. Clin. Invest. 90, 2197-2208.

Iwasaki A. and Medzhitov R. (2004). Toll-like receptor control of the adaptive immune responses. Nat. Immunol. 5, 987-995.

Jain R.K. (2001). Delivery of molecular medicine to solid tumors. lessons from *in vivo* imaging of gene expression and function. J. Control Release 74, 7-25.

Jain R.K. and Baxter L.T. (1988). Mechanisms of heterogeneous distribution of monoclonal antibodies and other macromolecules in tumors. significance of elevated interstitial pressure. Cancer Res. 48, 7022-7032.

Janeway Jr. C.A. (1989). Approaching the asymptote? Evolution and revolution in immunology. Cold Spring Harb. Symp. Quant. Biol. 54, 1-13.

Janeway Jr. C.A. and Medzhitov R. (2002). Innate immune recognition. Annu. Rev. Immunol. 20, 197-216.

Kang P.M. and. Izumo S. (2000). Apoptosis and heart failure. A critical review of the literature. Circ. Res. 86, 1107-1113.

Kannagi R., Izawa M., Koike T., Miyazaki K. and Kimura N. (2004). Carbohydrate-mediated cell adhesion in cancer metastasis and angiogenesis. Cancer Sci. 95, 377-384.

Karin M., Lawrence T. and Nizet V. (2006). Innate immunity gone awry: linking microbial infections to chronic inflammation and cancer. Cell 124, 823-835.

Kawai T. and Akira S. (2006). Innate immune recognition of viral infection. Nat. Immunol. 7, 131-137.

Kobata A. and Amano J. (2005). Altered glycosylation of proteins produced by malignant cells, and application for the diagnosis and immunotherapy of tumours. Immunol. Cell Biol. 83, 429-439.

Kuraya M. and Fujita T. (1998). Signal transduction via a protein associated with a glycosylphosphatidylinositol-anchored protein, decay-accelerating factor (DAF/ CD55). Int. Immunol. 10, 473-480.

Langman R.E. and Cohn M. (2002). If the immune repertoire evolved to be large, random, and somatically generated, then... Cell Immunol. 216, 15-22.

Lewis S.M. (1994). The mechanism of V(D)J joining. lessons from molecular, immunological, and comparative analyses. Adv. Immunol. 56, 27-150.

Lloyd K.O. and Old L.J. (1989). Human monoclonal antibodies to glycolipids and other carbohydrate antigens. dissection of the humoral immune response in cancer patients. Cancer Res. 49, 3445-3451.

MacPherson G., Kushnir N. and Wykes M. (1999). Dendritic cells, B cells and the regulation of antibody synthesis. Immunol. Rev. 172, 325-334.

Marchalonis J.J., Adelman M.K., Robey I.F., Schluter S.F. and Edmundson A.B. (2001). Exquisite specificity and peptide epitope recognition promiscuity, properties shared by antibodies from sharks to humans. J. Mol. Recogni. 14, 110-121.

Martin F. and Kearney J.F. (2001). B1 cells. similarities and differences with other B cell subsets. Curr. Opin. Immunol. 13, 195-201.

Medzhitov R. (2001). Toll-like receptors and innate immunity. Nature Rev. Immunol. 1, 135-145.

Medzhitov R. and Janeway Jr. C.A. (1997). Innate immunity. impact on the adaptive immune response. Curr. Opin. Immunol. 9, 4-9.

Medzhitov R. and Janeway Jr. C.A. (2000). The Toll receptor family and microbial recognition. Trends Microbiol. 8, 452-456.

Mekori Y.A. and Metcalfe D.D. (2000). Mast cells in innate immunity. Immunol. Rev. 173, 131-140.

Michel C.C. (1996). Transport of macromolecules through microvascular walls. Cardiovasc. Res. 32, 644-653.

Milenic D.E., Yokota T., Filpula D.R., Finkelman M.A., Dodd S.W., Wood J.F., Whitlow M., Snoy P. and Schlom J. (1991). Construction, binding properties, metabolism, and tumor targeting of a single-chain Fv derived from the pancarcinoma monoclonal antibody CC49. Cancer Res. 51, 6363-6371.

Milner E.C., Anolik J., Cappione A. and Sanz I. (2005). Human innate B cells: a link between host defense and autoimmunity? Springer Semin. Immunopathol. 26, 433-452.

Moretta L., Biassoni R., Bottino C., Mingari M.C. and Moretta A. (2002). Natural killer cells. a mystery no more. Scand. J. Immunol. 55, 229-232.

Mostov K.E. (1994). Transepithelial transport of immunoglobulins. Annu. Rev. Immunol. 12, 63-84.

Narula J., Pandey P., Arbustini E., Haider N., Narula N., Kolodgie F.D., Dal Bello B., Semigran M.J., Bielsa-Masdeu A., Dec G.W., Israels S., Ballester M., Virmani R., Saxena S. and Kharbanda S. (1999). Apoptosis in heart failure. release of cytochrome c from mitochondria and activation of caspase-3 in human cardiomyopathy. Proc. Natl. Acad. Sci. USA 96, 8144-8149.

Ochsenbein A.F. and Zinkernagel R.M. (2000). Natural antibodies and

complement link innate and acquired immunity. Immunol. Today 21, 624-630.

Ochsenbein A.F., Fehr T., Lutz C., Suter M., Brombacher F., Hengartner H. and Zinkernagel R.M. (1999). Control of early viral and bacterial distribution and disease by natural antibodies. Science 286, 2156-2159.

Papavasiliou F., Jankovic M., Gong S. and Nussenzweig M.C. (1997). Control of immunoglobulin gene rearrangements in developing B cells. Curr. Opin. Immunol. 9, 233-238.

Parkin J. and Cohen B. (2001). An overview of the immune system. Lancet 357, 1777-1789.

Pasare C. and Medzhitov R. (2005). Toll-like receptors. linking innate and adaptive immunity. Adv. Exp. Med. Biol. 560, 11-18.

Peng Y., Kowalewski R., Kim S. and Elkon K.B. (2005). The role of IgM antibodies in the recognition and clearance of apoptotic cells. Mol. Immunol. 42, 781-787.

Pohle T., Brändlein S., Ruoff N., Müller-Hermelink H.K. and Vollmers H.P. (2004). Lipoptosis. tumor-specific cell death by antibody-induced intracellular lipid accumulation. Cancer Res. 64, 3900-3906.

Prabhakar B.S., Saegusa J., Onodera T. and Notkins A.L. (1984). Lymphocytes capable of making monoclonal autoantibodies that react with multiple organs are a common feature of the normal B cell repertoire. J. Immunol. 133, 2815–2817.

Raulet D.H. (2004). Interplay of natural killer cells and their receptors with the adaptive immune response. Nat. Immunol. 5, 996-1002.

Rothenberg E.V. (2000). Stepwise specification of lymphocyte developmental lineages. Curr. Opin. Genet. Dev. 10, 370-379.

Silverman G.J. and Lucas A.H. (1991). Variable region diversity in human circulating antibodies specific for the capsular polysaccharide of Haemophilus influenzae type b. Preferential usage of two types of VH3 heavy chains. J. Clin. Invest. 88, 911-920.

Schatz D.G., Oettinger M.A. and Schlissel M.S. (1992). V(D)J recombination. molecular biology and regulation. Annu. Rev. Immunol. 10, 359-383.

Ternynck T. and Avrameas S. (1986). Murine natural monoclonal autoantibodies. a study of their polyspecificities and their affinities. Immunol. Rev. 94, 99–112.

Thomas L. (1959). In: Cellular and tumoral aspects of the hypersiitive states. Lawrence H.S. (ed). Hoeber-Harper. New York. pp 529 -532.

Thomas L. (1982). On immunosurveillance in human cancer. Yale L. Biol. Med. 55, 329-333.

Todorovska A., Roovers R.C., Dolezal O., Kortt A.A., Hoogenboom H.R. and Hudson P.J. (2001). Design and application of diabodies, triabodies and tetrabodies for cancer targeting. J. Immunol. Methods 248, 47-66.

Tonegawa S. (1988). Nobel lecture in physiology or medicine–1987. Somatic generation of immune diversity. In Vitro Cell Dev. Biol. 24, 253–265.

Turner G.A. (1992). N-glycosylation of serum proteins in disease and its investigation using lectins. Clin. Chim. Acta 208, 149-171.

Ulvestad E., Kanestrom A., Sonsteby L.J., Jureen R., Omland T., Edvardsen B., Lundervik J., Kristoffersen E. and van Dam A.P. (2001). Diagnostic and biological significance of anti-p41 IgM antibodies against Borrelia burgdorferi. Scand. J. Immunol. 53, 416-421.

Vlad A.M. and Finn O.J. (2004). Glycoprotein tumor antigens for immunotherapy of breast cancer. Breast Dis. 20, 73-79.

Vollmers H.P. and Brändlein S. (2002). Nature´s best weapons to fight cancer. Revival of human monoclonal IgM antibodies. Hum. Antibodies 11, 131-142.

Vollmers H.P. and Brändlein S. (2005a). Death by stress. natural IgM-induced apoptosis. Methods Find. Exp. Clin. Pharmacol. 27, 185-191.

Vollmers H.P. and Brändlein S. (2005b). The "early birds". natural IgM antibodies and immune surveillance. Histol. Histopathol. 20, 927-937.

Vollmers H.P., O'Connor R., Müller J., Kirchner T. and Müller-Hermelink H.K. (1989). SC-1, a functional human monoclonal antibody against autologous stomach carcinoma cells. Cancer Res. 49, 2471-2476.

Vollmers H.P., Dämmrich J., Ribbert H., Grassel S., Debus S., Heesemannn J. and Müller-Hermelink H.K. (1994). Human monoclonal antibodies from stomach carcinoma patients react with Helicobacter pylori and stimulate stomach cancer cells *in vitro*. Cancer 74, 1525-1532.

Vollmers H.P., Dämmrich J., Ribbert H., Wozniak E., Müller-Hermelink H.K. (1995). Apoptosis of stomach carcinoma cells induced by a human monoclonal antibody. Cancer 76, 550-558.

Vollmers H.P., Dammrich J., Hensel F., Ribbert H., Meyer-Bahlburg A., Ufken-Gaul T., von Korff M. and Muller-Hermelink H.K. (1997). Differential expression of apoptosis receptors on diffuse and intestinal type stomach carcinoma. Cancer 79, 433-440.

Vollmers H.P., Zimmermann U., Krenn V., Timmermann W., Illert B., Hensel F., Hermann R., Thiede A., Wilhelm M., Rückle-Lanz H., Reindl L. and Müller-Hermelink H.K. (1998a). Adjuvant therapy for gastric adenocarcinoma with the apoptosis-inducing human monoclonal antibody SC-1. first clinical and histopathological results. Oncol. Rep. 5, 549-552.

Vollmers H.P., Hensel F., Hermann R., Dämmrich J., Wozniak E., Gessner P., Herrmann B., Zimmermann U. and Müller-Hermelink H.K. (1998b). Tumor-specific apoptosis by the human monoclonal antibody SC-1. A new therapeutical approach for stomach cancer. Oncol. Rep. 5, 35-40.

Zitvogel L. (2002). Dendritic and natural killer cells cooperate in the control/switch of innate immunity. J. Exp. Med. 195, 9-14.

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