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Editorial

Is Rerum® the New Coley's Vaccine?

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Abstract: In this article I describe the development of an alternative type of immunotherapy that traces its origins to the nineteenth century when Dr. William B. Coley successfully treated inoperable cancer patients with his vaccine. Despite the well-documented successes, the vaccine did not survive Coley's times and was forgotten and neglected for decades in favor of radio- and chemotherapy. In the mid-fifties of the last century, the concept of a therapy based on stimulation of the immune system re-emerged from oblivion even though with a profoundly diverse connotation; the interest in this approach to cancer has grown exponentially ever since, up to the point of appointing immunotherapy of cancer the" breakthrough of the year" in 2013. Most cancer immunotherapies are based on proteins that either empower the immune system or attempt to selectively kill cancer cells. There are, however, some exceptions to this protein-based approach; the pioneering work of Dr. Prudden in New York and our research work in Florence, Italy. Thus, independently of each other, we pursued an alternative concept of immunotherapy that is based on glycosaminoglycans rather than proteins. From this research, a novel, tridimensional supramolecular structure constituted by chondroitin sulfate, vitamin D_3 and oleic acid, designated Rerum®, was developed with the intent of reviving the historical approach of Dr. Coley at the light of today's knowledge. Here, I describe the rationale for the development of Rerum® as well as some preliminary results that were presented at the Fourth International Congress of Integrative Medicine held in Fulda, Germany, in April 2017.

Keywords: Cancer, Immunotherapy, Vaccines, GcMAF, Autism

Introduction

Coley's Vaccine and Cancer Immunotherapy

Dr. William B. Coley is credited for having been the first to translate the observation of an association between bacterial infections, high fever and cancer remission into a successful cancer treatment. However, it is worth noting that such an association dates back to at least one century before Coley's times; as Wiemann and Starnes from the Department of Pharmacology of Amgen wrote in 1994 "As far back as the 1700s, it was recorded that certain infectious disease processes could exert a beneficial therapeutic effect upon malignancy" (Wiemann and Starnes, 1994). The so-called Coley's vaccine does not fit in today's definition of a cancer vaccine even though Coley's and today's *bona fide* vaccines have in common the goal of empowering the immune system so that it can fight cancer in a manner that is considered more physiological than many of the currently available anti-cancer therapies. Such an approach is nowadays designated "immunotherapy" and it has gone a long way since Coley's times. If in 1993 Pardoll of the Department of Oncology of Johns Hopkins wrote "active immunotherapy has not yet become an established modality of cancer therapy" (Pardoll, 1993), twenty years later, in 2013, Science heralded "Cancer Immunotherapy" as the breakthrough of the year dedicating to this topic the cover of the December 20 issue (Couzin-Frankel, 2013).

Takeuchi, a researcher of the Immunotherapy Division of the Kitatama Hospital in Tokyo, traced an interesting subdivision of modern immunotherapy in different phases or generations (Takeuchi, 1996). According to this Author, the beginning of modern immunotherapy, that is not counting Coley's experience, can be dated to 1953 with the observation of tumor specific transplantation antigens in animal models. These first attempts at immunotherapy lasted for about 30 years

and were characterized by an approach that was rather non-specific. After an initial burst of enthusiasm, however, this approach gradually lost appeal since the results were significantly below the over-emphasized expectations. Incomplete knowledge of the complex interplay between the immune system and cancer may have been one of the causes for the demise of the first generation of immunotherapy. More targeted approaches such as those using Cytotoxic T Lymphocytes (CTL) harvested from patients' cancers were developed as the next generation immunotherapy starting since 1986 and it is interesting to notice that the interest for immunotherapy has not waned ever since. In his paper, Takeuchi does not hesitate to define all the trials performed before 1953 as "nonsense"; however, he recognizes that such a definition of "nonsense" does not apply to Coley's experience by using the following words: "All trials were nonsense in the 0 (zero) generation before its birth except for Coley's vaccine" (Takeuchi, 1996).

It is safe to say that as of today the exact mode of action of Coley's vaccine remains somehow mysterious even though the role of high fever with consequent hyperthermia and the brutal stimulation of the immune system obviously played a major role. Such a role for high fever in fighting cancer is so well recognized that Hobohm, a researcher working at the famous pharmaceutical company Hoffmann-La Roche in Switzerland, in 2001 wrote: "A relationship between feverish infection and concurrent remission from cancer has been known about for a very long time" and "Fever induction under medical guidance may be considered as part of a therapy regimen for cancers of mesodermal origin" (Hobohm, 2001).

Since Coley injected mixtures of live, highly pathogenic, bacteria directly into inoperable tumors (McCarthy, 2006), a direct role for the microbes in fighting the cancer cannot be ruled out. Thus, it is well accepted that microbes such as Salmonella are endowed with the inherent capacity to colonize and eliminate solid tumors up the point that researchers from the Department of Experimental Therapeutics of the Beckman Research Institute of City of Hope in California, entitled a very recent review of theirs "Utilizing Salmonella to treat solid malignancies" (Ebelt and Manuel, 2017). The role of molecules pertaining to pathogenic microbes in the therapeutic effects of the Coley's vaccine was further highlighted in a paper published in 2012 by researchers working at the Section of Molecular Oncology and Immunotherapy in Rostock, Germany (Maletzki *et al*., 2012). In this publication, the Authors "systematically analyzed tumoricidal as well as immunostimulatory effects of the historical preparation Coley's Toxin, a safe vaccine made of heat-inactivated Streptococcus pyogenes and Serratia marcescens". They concluded that "Antitumoral effects following local therapy were primarily accompanied by stimulation of innate immune mechanisms" thus implying that molecules still active in the heat-inactivated microbial mixture were responsible for such an effect (Maletzki *et al*., 2012). An implication of these results that may have escaped the Authors' attention consists in the fact that the molecules responsible for these effects cannot belong to the class of proteins. Thus, heat inactivation of pathogenic bacteria also leads to denaturation of proteins; it is well known that temperatures above 41°C will break the interactions in many proteins and denature them. Therefore, independently of the role of the live bacteria present in the original mixtures used by Coley, the molecules responsible for the immunomodulatory, anti-cancer effects of the vaccine have to be found in another class of macromolecules that is notoriously resistant to heat. We hypothesize that this class of macromolecules is represented by glycosaminoglycans as it will be further elaborated in the following paragraphs.

Whatever the case, it is a historical fact that since 1891, for about forty years, Coley, in the quality of chief of the Bone Tumor Service at Memorial Hospital in New York, injected more than one thousand cancer patients with bacteria or bacterial products reporting excellent results in particular in sarcomas of bones and soft tissues. Such excellent results were replicated by other doctors and, by the turn of the century, the approach based on Coley's intuition was practiced on both sides of the Atlantic Ocean. However, as it often happens to pioneers in all fields of science, Coley's approach was severely criticized by his contemporaries who could not grasp the importance of the role of the immune system in fighting cancer; the concurrent development of other anti-cancer therapies that were easier to standardize, such as radio- and chemotherapy, led to the demise of the Coley's vaccine. Despite the fact that William B. Coley did not receive the recognition that he deserved in his times, today there is ample consensus that he deserves the title "Father of Immunotherapy" (McCarthy, 2006).

Quite obviously the current concept of immunotherapy is very different from that of the Coley's times when knowledge of the function of the immune system was rudimentary, to say the least, compared to the wealth of knowledge we enjoy today. Nevertheless, the basic notion that is shared by all those who try to define immunotherapy of cancer is that the goal of this approach is to empower the immune system so that it is the immune system itself that fights cancer at variance with radio- or chemotherapy that have the goal of directly killing the cancer cells and, in so doing, unavoidably, are responsible for the side effect of reducing the cancer fighting ability of the immune system. Therefore, immunotherapy "should be

understood as the activation of a whole system and not a pathway" (Bernichon *et al*., 2017) where in the concept of a "whole system" microbes of the human microbiota play an essential part. Thus, a very recent paper by researchers of the University of North Carolina describes how "a causative role - for microbes to cause cancer - is supported by rigorously controlled preclinical studies using gnotobiotic mouse models colonized with one or more specific bacteria" and, therefore, "Evidence is emerging that microbiota can be manipulated for improving cancer treatment" (Bhatt *et al*., 2017). Such a revaluation of the role of microbes in fighting cancer, albeit under a perspective completely different from that of Coley's, may strike a chord in those interested in the recursions of history.

An Alternative Approach to Immunotherapy; the Role of Glycosaminoglycans

If the basic notion or definition of immunotherapy is shared by researchers in the field, it is worth noticing that most, if not all, the approaches to immunotherapy involve the use of proteins, whether they are antibodies targeting specific cancer antigens (Zhang *et al*., 2017) or proteins known to modulate the immune system (Pacini *et al*., 2012). Oddly enough, immunotherapeutic approaches based on other macromolecules known to play a role in the immunology of cancer such as glycosaminoglycans, have not received much attention with two notable exceptions; our studies and the studies of Dr. John F. Prudden, a New York surgeon and researcher who had been using preparations made from animals' cartilage in the treatment of decubitus ulcers since the fifties of the last century.

We began working on the role of glycosaminoglycans in cancer in the early eighties, that is in a period defined by Takeuchi as the time of the first generation of immunotherapy (Takeuchi, 1996), when we demonstrated that glycosaminoglycans, then designated "heparin-like compounds", were involved in the negative regulation of the plasminogen activator. This is a pro-carcinogenic, pro-metastatic factor secreted by cells infected with the oncogenic SV-40 virus (Chiarugi *et al*., 1984). With a great leap of imagination one could have thought to translate those results *in vitro* in a novel form of immunotherapy of cancer based on glycosaminoglycans rather than on proteins that are molecules notoriously difficult to administer and use in clinical practice. As a matter of fact, that first paper published in the official journal of the Italian National Institute of Tumors, marked the beginning of a research effort that, some thirty years later, brought to the invention and development of Rerum®, arguably the most advanced form of immunotherapy based on glycosaminoglycans (Ruggiero, 2016). Thus, in the following two years, we demonstrated that glycosaminoglycans exert specific effects on platelets by remodeling the plasma membrane; we observed that drugs which inhibit the remodeling of the phospholipid bilayer of the platelet membrane abolished the effect of the glycosaminoglycans, whereas inhibitors of the enzyme cyclooxygenase were ineffective in this context (Ruggiero *et al*., 1984). These results were instrumental in the molecular design of the Rerum®, since this compound was developed to work by interacting with the plasma membrane of target cells.

Another milestone in understanding how glycosaminoglycans could be exploited in cancer immunotherapy came from our observation that, contrary to intuition, plasma glycosaminoglycans are associated with phospholipids and, in particular, with phosphatidylcholine, the phospholipid most represented in cell membranes (Vannucchi *et al*., 1985). This observation was rather puzzling because glycosaminoglycans are among the most hydrophilic molecules in the biological world and phospholipids are notoriously hydrophobic, their hydrophobicity being responsible for the self-assembly of cell membranes. We demonstrated that such an odd assembly of a circulating glycosaminoglycan with phosphatidylcholine could be explained by the non-covalent hydrophilic bonds established between the glycosaminoglycan and the polar head of phosphatidylcholine; interestingly these interactions occurred in a self-assembling manner that is without the intervention of enzymes or proteins and, therefore, independently of genetic information. It is worth considering that such an occurrence of molecular self-assembly in the absence of genetic information is thought to have been the first step in the origin of life (Mansy and Szostak, 2009). It is worth reflecting on the fact that molecules not pertaining to the flow of information in the classical interpretation of molecular biology since the times of Watson and Crick (DNA $\rightarrow$ RNA $\rightarrow$ Proteins) are assembled in our blood following a pattern as archetypical as life itself as we demonstrated in 1985 (Vannucchi *et al*., 1985).

In the same year 1985, Prudden published a paper describing excellent clinical results observed supplementing preparations of bovine cartilage to patients affected by different types of common cancers. The words used by Prudden to describe his observation were rather unusual for the style of scientific papers of those days and most likely reflected the enthusiasm for witnessing the unexpected recovery of cancer patients that had been labeled "incurable or inoperable". Thus, Prudden wrote: "Oral and subcutaneous administration of specific preparations of bovine tracheal cartilage rings (Catrix), a nontoxic agent, has resulted in a high response rate in 31 cases of a variety of clinical malignancies (response rate 90%, 61% complete). The demonstrated responder include present therapeutic

disasters such as glioblastoma multiforme and cancers of the pancreas and lung. Other types which were treated with success included cancers of the ovary, rectum, prostate, cervix, thyroid and an inoperable squamous cancer of the nose". Since Prudden was well aware of the need to identify the molecule(s) responsible for the observed clinical successes, he honestly concluded his paper with the words: "This wide range of Catrix efficacy now invites investigation by others to confirm the effectiveness of the material and to isolate the molecular entities responsible for these unexpectedly favorable results" (Prudden, 1985). In the years before his death in 1998 at the age of 78, Prudden published three more papers on this subject whose results were consistent with our approach to an immunotherapy of cancer based on glycosaminoglycans. Thus, in his last paper, Prudden identified a particular glycosaminoglycan, chondroitin sulfate, the same molecule that today is the backbone of Rerum®, as the compound responsible for the "unexpectedly favorable results" observed in incurable cancer patients and he hypothesized that such results were due to the "immunoaugmenting activity" of chondroitin sulfate; an unusual, but fundamentally correct, choice of words to indicate what we call today immunotherapy (Rosen *et al*., 1988).

The effects described by Prudden in cancer patients were consistent with our observation *in vitro* and in the experimental animal performed in the same years. Thus, in 1985 we demonstrated that heparin, a glycosaminoglycan with a molecular structure very similar to chondroitin sulfate, potentiated the anti-cancer effects of cortisone in transplantable murine cancers as diverse as melanoma, lung carcinoma and fibrosarcoma (Ziche *et al*., 1985). Quite clearly an effect on so diverse types of cancer implies the involvement of the immune system as a whole rather than a specific, chemotherapy-like, cancer killing effect. One year later, we demonstrated that heparin was internalized by cells in culture (Vannucchi *et al*., 1986). This observation bears important consequences in interpreting the results observed by Prudden in cancer patients as well as in the future development of Rerum®. Thus, consistent with our previous observation of a role for glycosaminoglycans in remodeling the cell membrane (Vannucchi *et al*., 1986), the mechanism of internalization implies that these molecules are able to interfere with the intracellular signaling mechanisms eventually leading to modulation of gene expression by interacting with DNA and/or with DNA-binding proteins. In other words, we can hypothesize two different mechanisms of action: A fast response due to the interaction and remodeling of the cell membrane and a long-lasting, sustained response due to the modulation of gene expression. It is interesting to notice that these two different types of responses were observed, about thirty years later, when Rerum® was used in the context of cancer (Schwalb *et al*., 2016).

The turning point in the search for the mechanism of action responsible for the role of glycosaminoglycans in a new form of immunotherapy, came a few years later when, in 1991, we were able to demonstrate that such complex, negatively charged, molecules were able to directly inhibit the proliferation of human epidermoid carcinoma cells in a model that is commonly used to study the cell cycle and the transmembrane signaling mechanisms peculiar of cancer (Vannucchi *et al*., 1991). Such a direct effect was independently confirmed several years later by other Italian researchers who, in a paper published in 2015, suggested that glycosaminoglycans, in that case, low-molecular weight heparin, improved survival of cancer patients by directly influencing the tumor biology (Franchini and Mannucci, 2015). Interestingly, we found out that the mechanism of action, at the cellular and molecular level, was different between normal, that is non-neoplastic and cancer cells. Thus, glycosaminoglycans, under certain conditions, are able to inhibit the abnormal proliferation of non-neoplastic smooth muscle cells and this feature may prove useful in the prevention of conditions such as atherosclerosis. One year before our observation of an effect on cancer cells, in 1990, we observed that high-molecular weight, but not low-molecular weight, heparins inhibited the proliferation of BC3H-1 smooth muscle cells *in vitro* and this effect was dependent on the binding of these long and negatively charged molecules to the cell membrane. Inhibition of cell proliferation was associated with inhibition of a particular transmembrane signaling mechanism that depends upon the metabolism of inositol phospholipids (Vannucchi *et al*., 1990). However, such a membrane remodeling effect was not at work in the inhibition of cancer cell proliferation, thus lending credit to the hypothesis that the mechanism of action involved in the anti-cancer effect of glycosaminoglycans was to be found in the internalization of the molecules and in their interaction with DNA and/or DNA-binding proteins with consequent changes of gene expression. Such a selective mode of action was further confirmed at the molecular level by a subsequent study of ours published in 1993, where we demonstrated that a glycosaminoglycan, heparin, worked selectively on cancer cell signaling without affecting normal signaling even when the signaling was within the same framework of intracellular communication. More specifically, we demonstrated that heparin inhibited proliferation and signaling induced by the Platelet-Derived Growth Factor (PDGF) in cancer cells transformed by the oncogene *sis* that is the oncogene coding for PDGF. However, in normal, non-neoplastic, cells, heparin did not affect the signaling of the same growth factor PDGF as if the anti-oncogene effect of

the glycosaminoglycan were selective for cancer cells (Cavari *et al*., 1993). These results were of utmost importance for the future molecular design of Rerum® because they provided the first experimental evidence that we could selectively target cancer cells without interfering with normal cell functions, an effect, a sort of "Holy Grail", that has been sought after since the beginning of the fight by humans against cancer.

The Development of a Novel Approach to Glycosaminoglycan-Based Immunotherapy

It was based on these data obtained at the molecular level that, in 2015, I embarked in the quest for a non-protein-based approach to immunotherapy that led to the formulation of Rerum®. Rerum® is a proprietary emulsion of chondroitin sulfate, vitamin D_3 and oleic acid bound together in a tridimensional array of hydrophobic and hydrophilic interactions. The rationale for the design of Rerum® is described in a paper published in 2016 in the journal Medical Hypotheses (Ruggiero *et al*., 2016) and in an editorial on the Gc protein-derived Macrophage Activating Factor (GcMAF) and autism published shortly thereafter in this Journal (Ruggiero, 2016). The reason why Rerum® could be considered the new Coley's vaccine lays in the consideration that Coley's vaccine was a rudimentary, albeit tremendously efficient, combination of immunotherapy and direct anti-cancer effects that is replicated, in a more controlled and safe manner, by Rerum®. Thus, each one of the molecules constituting Rerum® is endowed both with immunomodulating and anti-cancer properties that, in many cases have been known for centuries as it was described for oleic acid, jokingly dubbed "... anti-oncogenes nutraceuticals since the 17th Century" (Maciag *et al*., 2008). Although the immunomodulating and anti-cancer properties of chondroitin sulfate, vitamin D_3 and oleic acid have been known for, at least, decades, Rerum® adds a novel feature that is due to the assembly of the three molecules in one single tridimensional supramolecular structure. Thus, being assembled in a single structure, the three molecules target the same cell at the same time and in the same constant molecular combination; this means that one molecule of chondroitin sulfate, that typically encompasses 50-100 repetitive units of N-acetylgalactosamine (the active site of GcMAF) and glucuronic acid, is bound to a precise number of vitamin D_3 and oleic acid molecules and the ratio between the three molecules remains constant. Conversely, if the three molecules constituting Rerum® were independently administered as three distinct supplements, each target cells would receive, at different times, a different number of each molecule. Therefore, based on this simple calculation, we can hypothesize that the biological and clinical effects of Rerum® are greater than the simple sum of the biological and clinical effects of each molecule constituting it.

Since Rerum® is constituted by known supplements that have been used for decades and are recognized as safe, it falls in the category of supplements and it is registered as such in the European Union and Switzerland. Although supplements are usually meant to be administered through the oral route, in compliance with national rules and regulations and under the direct responsibility of the Therapist, they can also be administered through other routes such as the sublingual or the parenteral. The subcutaneous, intradermal or intramuscular routes of administration are of particular interest since these are efficient ways to prime and modulate the immune system in the context of vaccinations and immunotherapies (Herzog, 2014; Casale and Stokes, 2014) and are consistent with the observation of Prudden who reported excellent results using a combination of oral and subcutaneous administration of bovine cartilage extracts (Prudden, 1985). Using a variety of routes of administration, Rerum® has been used in the complementary approach to cancer in patients with different malignancies; preliminary results concerning this clinical experience were published in this Journal in 2016 (Schwalb *et al*., 2016).

Following the publication of those preliminary results in 2016, further observation using this novel type of immunotherapy reminiscent of the approaches of Coley and Prudden, were presented at the Fourth International Congress on Integrative Medicine that was held in Fulda, Germany, on April 1 and 2, 2017. Here, Medical Doctors and Therapists from different countries described the results they had observed using this glycosaminoglycan-based immunotherapy in a number of different conditions ranging from cancer to autism, from pain to persistent Lyme disease. Of particular interest in this context were the results observed *in vitro* by Drs. Siniscalco and Brigida working at the University of Campania "Luigi Vanvitelli". The Italian researchers observed that Rerum® showed a selective anti-proliferative effect against adenocarcinoma human cells derived from cancerous lung tissue and induced the apoptosis of the cancer cells through a caspase-dependent mechanism. The selectivity of effects was demonstrated by studying in parallel normal human embryonic kidney cells that, at variance with cancer cells, were protected by the compound. These results are consistent with our old observation that glycosaminoglycans exert opposite effects in normal and cancer cells (Cavari *et al*., 1993), thus being excellent candidates for a novel type of immunotherapy that is based on these macromolecules instead of proteins. In addition to these considerations regarding the glycosaminoglycan moiety of Rerum®, it should be noticed that also the fatty acid (oleic acid) moiety of the

compound shows selective anti-cancer properties. Thus, it has been demonstrated that the molecule responsible for the selective cancer killing properties of Human Alpha-lactalbumin Made LEthal to Tumor cell (HAMLET) is indeed oleic acid rather than the protein lactalbumin (Jung *et al*., 2016) that functions solely as a carrier. In the case of Rerum® the carrier of oleic acid is chondroitin sulfate, a molecule that, unlike lactalbumin, is intrinsically endowed with anti-cancer, immunomodulating properties that contribute to the overall efficacy of the approach.

Although the complex and controversial topic of autism, vaccines and the immune system is well beyond the scope of this article, it is worth mentioning that Dr. Antonucci, a psychiatrist and researcher working at the Biomedical Centre for Autism Research and Treatment in Italy, with remarkable experience in immunomodulating treatments and autism (Siniscalco *et al*., 2014), speaking at the Congress in Fulda, reported a response rate of about 80% observed treating autistic children with Rerum®. Such results lend credit to the hypothesis that this novel type of immunotherapy may be useful in a number of conditions associated with immune system dysfunction.

Conclusion

If the history of immunotherapy can be divided in phases or generations as proposed by Takeuchi (1996), then our approach based on glycosaminoglycans could be classified as second generation immunotherapy since it was born in the mid-eighties when, independently of each other, Prudden in New York and our research group in Florence, Italy, begun to describe the anti-cancer, immunomodulatory effects exerted by these molecules in a variety of *in vitro* and *in vivo* models. We feel, however, that such a strict classification based on temporal criteria does not apply to the novel approach to immunotherapy described in this article. Thus, the approach based on Rerum® combines old observation on the role of chondroitin sulfate, vitamin D_3 and oleic acid in immunotherapy with innovative concepts based on a detailed knowledge of their mode of action at the molecular level. We feel that Rerum® addresses the invitation by Prudden in his paper of 1985 when he wrote "This wide range of Catrix efficacy now invites investigation by others to confirm the effectiveness of the material and to isolate the molecular entities responsible for these unexpectedly favorable results" (Prudden, 1985).

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Author's Contribution

The Authors contributed to the genesis and development of the project described in this study and to the writing of the manuscript.

Ethics

This article is original and contains unpublished material.

Conflict of Interest

Marco Ruggiero, MD, PhD, is the inventor and the owner of the intellectual property of Rerum® that is licensed to dr. reinwald healthcare gmbh + co kg, Germany. Marco Ruggiero is the founder and CEO of the Swiss company Silver Spring sagl, a company that produces and distributes foods and supplements; none of the products of this company is mentioned in this article. Marco Ruggiero is member of the Editorial Board of The American Journal of Immunology and is waived from the Article Processing fee for this contribution; he receives no remuneration for his editorial work.

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