agreements is only a ceremonial first step; they must subsequently be ratified and strengthened over time[5]. I believe that Farman and colleagues' paper led to the remarkably fast ratification of the protocol in 1989, and to later amendments (beginning with the London Amendment in 1990) that included ever-tightening restrictions on the global production and consumption of ozone-depleting substances.

So why was the ozone hole not seen in computational simulations of the stratosphere? It turned out that the models lacked a key ingredient: by considering only gas-phase atmospheric chemistry, they overlooked the activation of ozone-destroying chlorine species that occurs on and within polar stratospheric cloud particles at extremely low temperatures[11,12]. The discovery of the missing ingredient drew physical chemists in increasing numbers to study the surface chemistry involved[13]. Previously unknown gas-phase reactions associated with ozone depletion were also identified, particularly those involving a ClO dimer (see ref. 10, for example). Laboratory and field studies were carried out, and microphysical models were developed (see ref. 14, for example), to determine what polar stratospheric clouds are made of: ice, nitric acid hydrates or supercooled liquids. The answer was that they could be all three, depending on temperature and the histories of the sampled air parcels.

Ground-based and airborne missions to understand Arctic ozone chemistry[15] were also inspired by Farman and colleagues' paper and related studies. It emerged that ozone loss in the Arctic is generally much less severe than in the Antarctic, broadly because temperatures in the region are warmer as a result of meteorological differences between the two regions. The coupling of chlorine-containing species with bromine-containing ones was found to be a key ingredient in polar ozone depletion, especially in the Arctic[16].

Atmospheric modelling also progressed to simulate the newly discovered processes, evolving from two dimensions (latitude–altitude) to three (latitude–altitude–longitude), to better represent global stratospheric temperatures, winds and circulation[17]. Dynamical studies have shown that the ozone hole influences Antarctic winds and temperatures not just in the stratosphere, but also in the underlying troposphere, and there is evidence for climate connections at other latitudes[18]. Modern global climate models therefore include increasingly detailed representations of stratospheric chemistry and dynamics. The ozone hole has thus inspired a new generation of scientists to probe climate–chemistry interactions, forging connections between previously separate disciplines.

The Montreal Protocol led to global CFC production and consumption phase-outs by 2010, and now the Antarctic ozone hole is slowly healing[10]. The protocol thus prevented the ozone layer from collapsing[19] and is a signature success story for global environmental policy. Because CFCs have atmospheric lifetimes of 50 years or more, the atmosphere will not fully recover until after 2050, even in the absence of further emissions.

However, recent work[20] provides strong evidence of the continuing production and release of one type of CFC (trichlorofluoromethane). The source is not large enough to reverse the healing of the ozone hole, but it is slowing recovery and shows that there is still a need for scrutiny in this field. Research into, and policy to protect, the stratosphere will thus continue to be inspired by Farman and colleagues' research — and will probably do so until the ozone hole finally closes.

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Immunology

The advent and rise of monoclonal antibodies

Klaus Rajewsky

A 1975 *Nature* paper reported how cell lines could be made that produce an antibody of known specificity. This discovery led to major biological insights and clinical successes in treating autoimmunity and cancer.

In their 1975 *Nature* paper[1], the immunologists Georges Köhler and César Milstein described the production of monoclonal antibodies of predetermined specificity, each made by a continuously growing cell line that had been generated by the fusion of an antibody-producing cell from an immunized mouse with an immortal cancer cell specialized for antibody secretion. Hearing from César about this work before it was published, on the way to an obscure meeting in San Remo in Italy, I knew immediately that our research field had reached a turning point.

Antibodies were discovered in 1890 by the physiologist Emil von Behring and the microbiologist Shibasaburo Kitasato as protective antitoxins in the blood of animals exposed to diphtheria or tetanus toxin[2]. Ever since, antibodies have been a major research subject, given their key role in adaptive immunity (specific immune responses against, for example, invading disease-causing agents) and their wide range of specificities, essentially covering the universe of chemical structures. This had stood out from early on as a major genetic puzzle. How can our limited genome encode a seemingly limitless repertoire of specificities? And in medical (and industrial) practice, antibodies have been used ever since their discovery as the basis for serum therapy (the treatment of infectious diseases using blood serum from immunized animals), as diagnostic tools to monitor infectious disease, and in innumerable other contexts.

But antibodies specific for any given molecule (called an antigen in the context of an antibody response) came, with a few notable exceptions, as mixtures of antibodies, produced by thousands of antibody-producing cells in an immunized animal or infected person. Each of these cells produced an antibody of its own kind, so that 'antibody specificity' usually referred to the properties of antibody populations rather than those

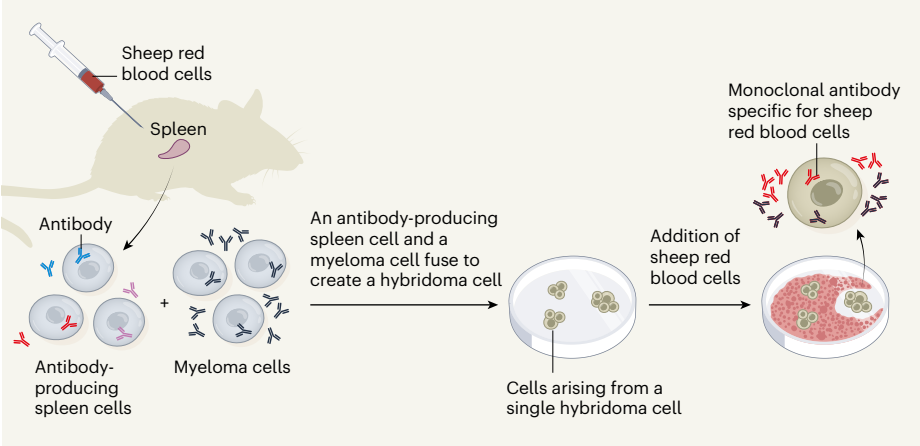

Figure 1 | The production of monoclonal antibodies. Köhler and Milstein's 1975 *Nature* paper[1] solved the problem of how to generate clones of continuously dividing cells that make antibodies of a known specificity. The ability to generate such monoclonal antibodies revolutionized antibody research and paved the way to clinical advances. The authors injected mice with sheep red blood cells and isolated spleen cells, including those that produce antibodies. Different antibody colours indicate antibodies specific for different molecules (antigens), and produced by different cells. The authors had the idea of fusing antibody-producing spleen cells of limited lifespan with myeloma cells – immortal cancerous immune cells secreting antibodies of unknown specificity. Spleen cells that had been activated upon antigen recognition fused preferentially with the myeloma cells, generating hybrid cells called hybridomas. Unlike unfused cells, the hybridoma cells could grow on the selective agar plates used, and formed colonies of identical cells. Hybridomas that secreted antibodies specific for sheep red blood cells were identified by their ability to destroy such cells when added to the agar, generating a clearance (plaque). These original hybridoma cells made two types of antibody, one that recognized sheep red blood cells and another of unknown specificity.

of individual antibodies. The inability to produce molecularly defined, homogeneous antibodies of predetermined specificity was a major hurdle that needed to be overcome.

This changed overnight with Köhler and Milstein's paper. Köhler had joined Milstein's group at the MRC Laboratory of Molecular Biology in Cambridge, UK, as a postdoc, to study the mechanism of somatic mutation that operates in antibody diversification. The plan was to use mouse myeloma cells for this purpose. These are tumour cells originating from antibody-secreting immune cells. The cancer immunologist Michael Potter at the National Cancer Institute in Bethesda, Maryland, had shown years before that myelomas could be induced in a particular mouse strain by the injection of mineral oil[3]. The Milstein team was propagating and fusing to each other cells obtained from cell lines derived from various such tumours. However, the myeloma antibodies were ill-defined in terms of specificity. Could one perhaps fuse antibody-producing cells from immunized mice to myeloma cells, to produce continuously dividing cells that make antibodies specific for the immunizing antigen? To detect such fused cells, an approach offered itself which Köhler had become acquainted with during his PhD at the Basel Institute for Immunology in Switzerland and that had been developed by the institute's director, Niels Jerne[4]. This was a simple technique in which cells secreting antibodies in response to, and specific for, sheep red blood cells (SRBCs) can be identified by the formation of a clearance (called a plaque) in SRBC-containing agar plates.

With this, the stage was set for the Köhler–Milstein experiment (Fig. 1). Large numbers of plaque-forming hybrid cells secreting anti-SRBC antibodies appeared when spleen cells from SRBC-immunized mice were fused with myeloma cells. The fused cells had acquired expression of a single type of anti-SRBC antibody from a spleen cell and preserved the immortality and high rate of antibody secretion of the myeloma fusion partner. Myeloma and spleen cells were unable to multiply under the chosen experimental conditions, and the myeloma cells apparently preferred antigen-activated spleen cells over others for fusion, a prerequisite for the striking success of the experiment.

The fused cells could be cloned and propagated indefinitely as what were later termed hybridomas, producing unlimited amounts of monoclonal antibodies. The first-generation hybridomas secreted two types of antibody: the desired one, plus an antibody of unknown specificity originating from the myeloma fusion partner. But this two-antibody problem was soon solved through the isolation of myeloma lines that had lost antibody expression[5,6].

Antibodies against any desired antigen could now be generated, investigated and used as homogeneous molecular entities. In 1984, Köhler and Milstein won the Lasker Award together with Potter, and that same year Köhler, Milstein and Jerne were awarded the Nobel Prize in Physiology or Medicine.

The impact of the Köhler–Milstein paper on biomedical and, specifically, immunological research was dramatic, propelled by scientific developments that occurred around the time the paper appeared. Thus, it became clear shortly afterwards that the variable and constant regions of antibodies are encoded by separate gene segments. Antibody diversity arises when somatic recombination joins gene segments together, and when a subsequent process called somatic hypermutation operates, during the course of the antibody response, on the recombined gene segments encoding antibody variable regions. Together, these mechanisms generate a vast repertoire of antibody specificities, as well as distinct classes of antibody, which mediate their various roles (effector functions) through their differing constant regions.

These insights were accompanied by the explosive development of new molecular and genetic tools that allowed the isolation and manipulation of antibody genes in multiple ways. Together with the hybridoma technology, they fuelled a rapidly growing and still expanding field of investigation, in which basic research on antibody diversification and effector function goes hand-in-hand with the production and engineering of monoclonal antibodies for diagnostic and therapeutic purposes.

In the early days, the production of monoclonal antibodies was entirely based on hybridoma technology and used for two main purposes: to study the somatic evolution of the antibody repertoire and the molecular basis of antibody specificity; and to generate reagents that bind to specific proteins or other molecules expressed by cells of the body or by pathogens. In both cases, completely new insights and technical advances resulted. Thus, affinity maturation of antibodies (the increase of antibody affinity during the course of an antibody response) began to be understood at the molecular level. And the technique of fluorescence-activated cell sorting was revolutionized by monoclonal antibodies, allowing the separation of different cell types at an unprecedented level of specificity and resolution. Recent highlights in this area include approaches allowing gene-expression profiling of single cells that have been characterized by the expression of large arrays of surface-marker proteins through cocktails of DNA-tagged, 'barcoded' monoclonal antibodies[7].

In medicine, monoclonal antibodies have an ever-increasing role and have generated a multibillion-dollar market, which is expected to grow substantially in the future. In addition to their impact on medical diagnosis, the therapeutic application of antibodies has led to spectacular successes in the treatment of autoimmune diseases and cancer. The 2018 Nobel Prize in Physiology or Medicine was awarded for the "discovery of cancer therapy by [antibody-mediated] inhibition of negative

immune regulation". As often happens in biology, both the mechanisms and the efficient induction of the inhibitory processes underlying this type of immunotherapy are still unclear, with ongoing research providing challenges and new perspectives that are driving the development of monoclonal antibodies against additional targets.

Monoclonal antibodies are also being developed to control infectious diseases – following the concept of protective antibodies that goes back to von Behring and Kitasato. Prevalent diseases such as malaria, influenza and AIDS call for the development of what are termed broadly neutralizing monoclonal antibodies, which, applied individually or in cocktails, might provide broad protection[8].

Intensive work in this direction has yielded promising results, including engineering antibody specificity through the substitution of variable domains by ligand-binding domains from non-antibody receptors[9]. Yet the immune system itself uses similar tricks[10] and, by and large, antibody design is still unable to outdo it in terms of generating and selecting antibody specificities[11]. Nevertheless, the manifold modern molecular, cellular and genetic approaches to selecting and engineering antibodies have had, and continue to have, a tremendous impact on the field, whether by producing partly or fully human antibodies of different classes, making bi-specific or toxin-conjugated antibodies for specific therapeutic purposes, or incorporating antibody variable regions into chimaeric antigen receptors on T cells for use in an anticancer treatment called CAR-T cell therapy.

Monoclonal antibodies are nowadays often generated by isolating or transforming antibody-producing cells taken directly from immunized animals or patients, and transplanting the antibody-encoding genes of these cells into suitable producer cell lines, rather than using hybridoma technology[12–14]. But they started their spectacular career in 1975, secreted by hybridoma cells in Köhler and Milstein's SRBC-containing agar plates.

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Materials science

The nano-revolution spawned by carbon

Pulickel M. Ajayan

In 1985, scientists reported the discovery of the cage-like carbon molecule C_{60}. The finding paved the way for materials such as graphene and carbon nanotubes, and was a landmark in the emergence of nanotechnology.

The history of the carbon molecule C_{60} highlights the fact that discoveries do not happen in a predefined sequence. C_{60}, carbon nanotubes and graphene (single layers of graphite) are essentially members of the same family: all are nanoscale structures that consist of carbon atoms arranged in a periodic crystal lattice. Graphite has been known for a few hundred years, and individual layers of the material could be separated easily. However, the identification of C_{60} by Kroto *et al.*[1] did not occur until 1985. This, in turn, led to the discovery of graphene nearly two decades later[2]. Both of these breakthroughs led to Nobel prizes, in chemistry for C_{60} (1996) and in physics for graphene (2010).

The discovery of C_{60} occurred on the campus of Rice University in Houston, Texas. Eiji Osawa, a Japanese theoretical chemist, had predicted[3] the stable structure of a 60-atom carbon molecule in 1970, but this finding did not come to the attention of the mainstream scientific community. Experimental results from mass spectrometry were also beginning to emerge, showing the stability of 60-atom carbon clusters. However, no one made the connection that these clusters would have the structure that Osawa had predicted. It

150 years ago

Aphorisms by Goethe — the opening article of the first issue of *Nature*, 4 November 1869.

Nature! We are surrounded and embraced by her: powerless to separate ourselves from her, and powerless to penetrate beyond her. Without asking, or warning, she snatches us up into her circling dance, and whirls us on until we are tired, and drop from her arms. She is ever shaping new forms: what is, has never yet been; what has been, comes not again. Everything is new, and yet nought but the old ...
So far Goethe.

When my friend, the Editor of NATURE, asked me to write an opening article for his first number, there came into my mind this wonderful rhapsody on "Nature", which has been a delight to me from my youth up. It seemed to me that no more fitting preface could be put before a Journal, which aims to mirror the progress of that fashioning by Nature of a picture of herself, in the mind of man, which we call the progress of Science.

[*In a letter to Chancellor von Müller*] Goethe says, that about the date of this composition of "Nature" he was chiefly occupied with comparative anatomy; and in 1786, gave himself incredible trouble to get other people to take an interest in his discovery, that man has a intermaxillary bone. After that he went on to the metamorphosis of plants; and to the theory of the skull; and, at length, had the pleasure of his work being taken up by German naturalists. The letter ends thus :—"If we consider the high achievements by which all the phenomena of Nature have been gradually linked together in the human mind ... we shall, not without a smile ... rejoice in the progress of fifty years."...

When another half-century has passed, curious readers of the back numbers of NATURE will probably look on *our* best, "not without a smile;" and, it may be, that long after the theories of the philosophers whose achievements are recorded in these pages, are obsolete, the vision of the poet will remain as a truthful and efficient symbol of the wonder and the mystery of Nature.

T. H. Huxley