The birth of LDL-apheresis (2009)

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20. Anniversary of Milenyi Biotec **August 14, 2009**

It is my pleasure to participate in these celebrities on the occasion of the 20. anniversary of Miltenyi Biotech and contribute with a short retrospective.

The view in Biotechology today is directed towards future developments. However in the hectic world of Biotechnology it might be worthwhile sometimes to halt, and reflect the past and learn from the pathway, which led to the achievements of the past.

I try to verbalize personal feelings coming from the heart and report from the laboratory about an episode of my long scientific life, namely the origin of immune apheresis. It was a period full of exciting experiments and results, with happy and frustrating hours and peculiarities along with this research project which began three decades back. This project developed to a paragon of translational medical research.

A few years ago, you might remember this, dear Stephan, we met and you mentioned rather emotionless that you have come close to my past interests. I learnt that you had acquired the facilities in Teterow, a modern plant, which was devoted to manufacture anti- LDL affinity columns for clinical application in the treatment of hypercholesterolemic patients and that you planned to continue your engagement in the immuno-apheresis in development and production. I was very happy about your decision to follow up this potent new

approach. You decided to do so, despite there was no patent protection, which I missed because of my deficit in economic.
We will hear later that our paradigm of LDL-immuno-apheresis as a therapeutic principle, expanding to a growing number of medical indications.
Ever since I heard that a Diploma student of Andreas Radbruch in the Department of Genetics, Stephan Miltenyi, started a laboratory in a garage to pursue his ideas about magnetic cell separation, I followed your admirable career. I am aware of your modesty. But today, two decades of your life as scientist, economic leader, head of a company with highest standards in biotechnological research and development with worldwide recognition, you should be very proud.
Let me also express my deep satisfaction about the fusion with former MEMOREC. You have recognized the great scientific and highly organized potential of MEMOREC with its bright and creative brains, highly competent, engaged and loyal personalities. This fusion of Molecular biology and immunology lends Miltenyi an ideal profile to serve biomedical research.
It is rewarding to see that Miltenyi is not endangered by shareholders and bankers. And I sincerely hope that thoughtful, wise and prospective decisions in the future will guarantee a continuing procreative productivity and development of your institution.

I will restrict my report to the early development of LDL apheresis, which had an eventful past, with amazing events and curiosities.

As a disposition of this review I take the abstracts of our two original publications in PNAS 1981, together with a medical student, Thomas Demant and of the Lancet paper with Volker Greve and Helmut Borberg from the Department of Internal Medicine of our faculty, who was intrumental in the introduction into the clinical application.

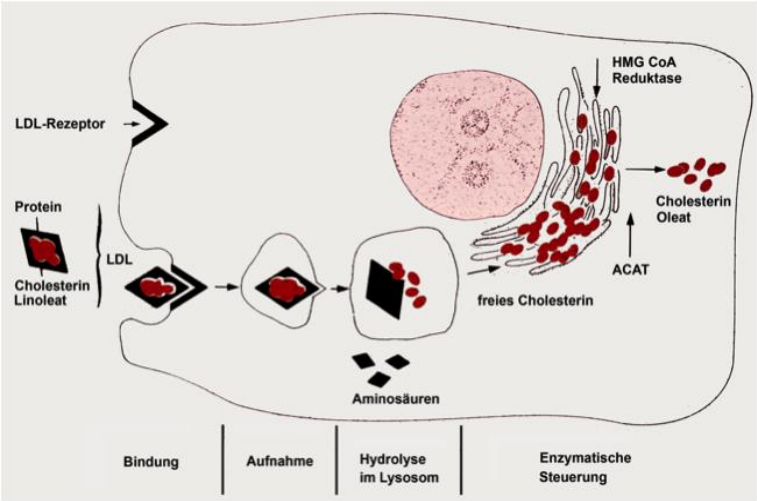
The initial publication ends with the perspective: *This continuous plasma separation-immuno-adsorption procedure may have broad applicability for the elimination of any plasma component with antigenic properties.* And indeed it is expanding into hematology, rheumatology, cardiology, nephrology, neurology, gastroenterology and transplantation medicine.

Abstracts report cold facts. They leave no space for the thrill, fun, frustration and the silent pride of the experimenter. But these are the ingredients, the spices of research and therefore I will feed in some of them, which we had during this project.

In the seventies my laboratory was studying protein lipid interactions in membranes and serum lipoproteins and their importance for assembly and function, in particular of High and low density lipoproteins, HDL and LDL. Lipoproteins were so fascinating, because they were easily available and rather simple in protein and lipid composition. Furthermore they gave insight into cholesterol and triglyceride transport in the circulation and, last but not least, provided the key to our understanding of lipid and lipoprotein metabolism with hypercholesterolemia as the most important because of its relevance for atherosclerosis. Cholesterol is essential for membrane integrity but pathogenic, when present in elevated

serum concentrations, which causes atherosclerotic plaques and coronary heart disease.

At this time Goldstein and Brown had developed the LDL receptor concept and its genetic defect as the basis for the familiar hypercholesterolemia, FHII. Soon after Mevinolin was recognized as the first HMG-CoA reductase inhibitor to block cholesterol synthesis by Endo in Japan, and later



developed to the group of statines, which were immediately heavily propagated for the treatment of hypercholesterolemia, it became clear, that the statins alone were ineffective in the treatment of this severe genetic disease.

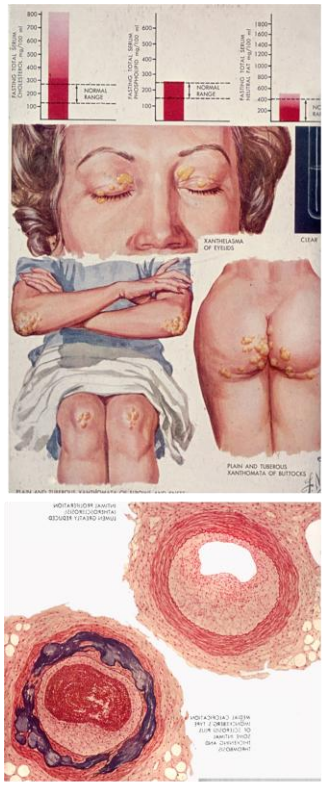
The Low density lipoprotein (LDL)-receptor concept

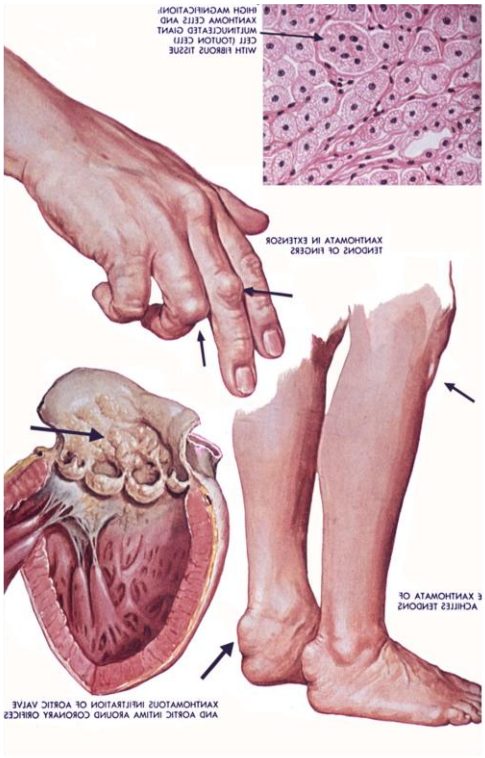
Familial hypercholesterolaemia: a genetic receptor defect

atherosclerosis

cholesterol-lowering procedures:

Medication and/or elimination?





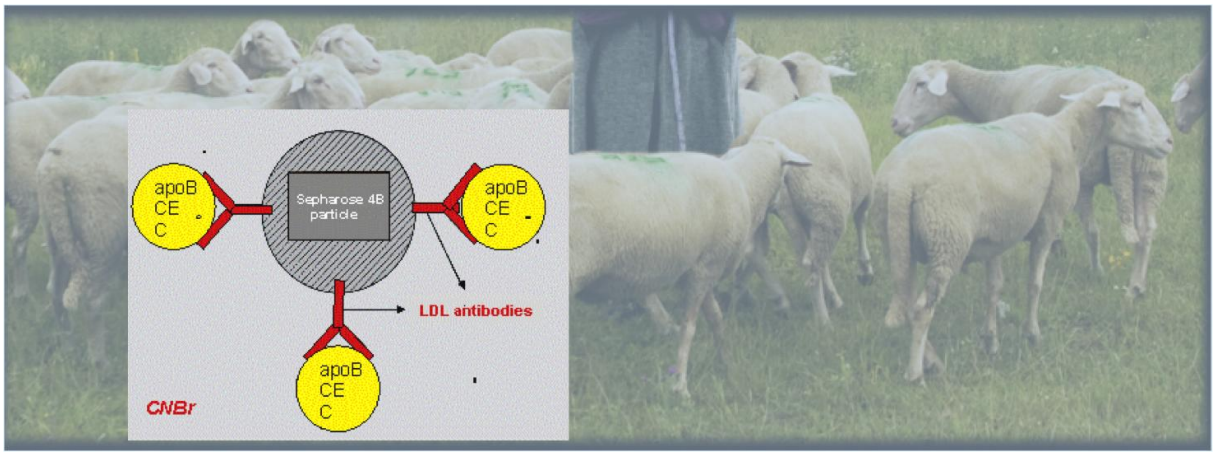
Familial hypercholesterolemia type II

Just at this time a medical student, Thomas Demant, asked for doing his medical thesis in my laboratory. I decided that Thomas Demant should neither be challenged by a sophisticated biochemical and biophysical topic within our ongoing research nor follow the main stream of studies in these days on the inhibition of cholesterol biosynthesis with inhibitory drugs, but rather elaborate initial prerequisites of a new concept, namely the selective elimination of the undesirable excess blood plasma carrier of cholesterol, the apoB containing lipoproteins VLDL and LDL. Thomas should use immune absorption, a long standing biochemical tool, and quantify the efficacy of the removal of VLDL and LDL lipoprotein particles from plasma with anti apoB antibodies by a solid phase techniques, apoB or LDL, covalently linked to a solid phase (Sepharose4B beads. First, measure the elimination kinetics in vitro from pig

and human plasma, then transfer the method to an in vivo experiment in the Minnesota mini pig and finally in vivo in human FH patients in the extracorporeal circulation.

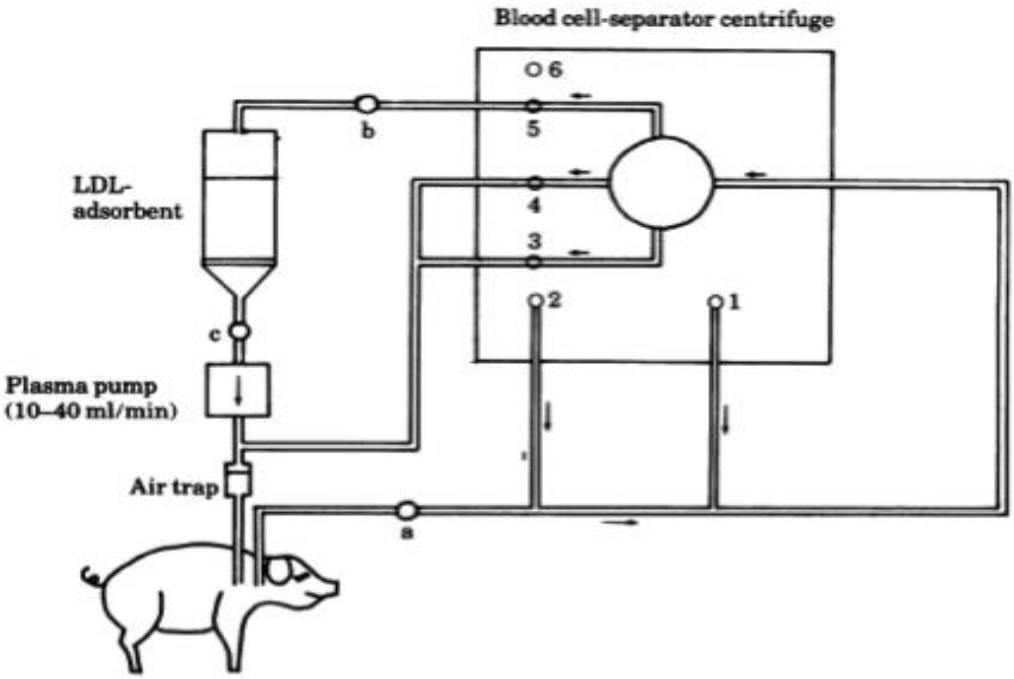
I will briefly outline the steps required which are based on the principle of specificity and strength, governing antigen-antibody interactions.

1. Immunization of sheep with purified swine and human LDL (apoB)
2. Chemical coupling of swine and human apoB antigen (VLDL and LDL) to Sepharose 4B.
3. Design of Sepharose 4B columns with optimal rheologic properties for maximal absorption (antigen column)
4. Selective mass antibody isolation from sheep serum of
   a) anti-pig and
   b) anti-human LDL-antibody



Chemical coupling of monospecific polyclonal anti apoB specific antibodies to Sepharose4B beads for affinity chromatography (antibody column)
Effective removal in vitro of pig and human plasma LDL by anti-pig and anti human LDL-Sepharose affinity chromatography

5. Proof of principle in the hypercholesterolemic mini pig



The first mini pig Paula was fed a high cholesterol Western diet and Paula was grateful

a) to develop a severe coronary atherosclerosis,

b) to allow us to set up of the experimental design for the extracorporeal immuno-adsorption.

We used first plasma separator membranes, but then preferred an outdated blood cell separator from the Department of

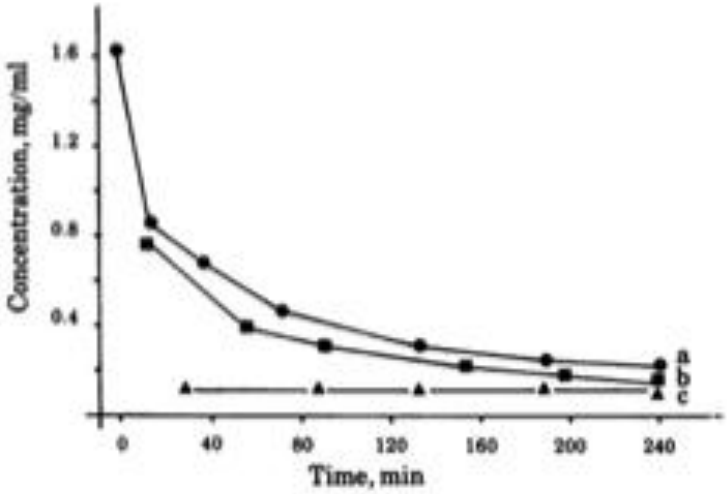


FIG. 2. Kinetics of LDL removal from pig plasma *in vivo* by adsorption to anti-LDL-Sepharose CL-4B, showing plasma LDL concentration before plasma separation (curve a) and LDL concentration before (curve b) and after (curve c) LDL adsorption.

Medicine. We had to learn sophisticated techniques, e.g. we were instructed by a young surgeon to apply an a. carotis-v. jugularis shunt, tunneled to the neck.

Thomas recorded beautiful LDL elimination kinetics of Paula.

Paula also allowed repeated coronary angiography by a young cardiologist. We preferred to proceed the short way, in German: “den unteren Dienstweg gehen”. But this was no easy task.

As a side remark, we had neither acceptance nor support from the heads of the Clinical departments. At that time VLDL, LDL and HDL and the Fredrickson lipoprotein scheme were strange acronyms for many German Cardiologists. It was therefore not surprising that my grant application with the DFG was turned down by the clinical reviewers, but

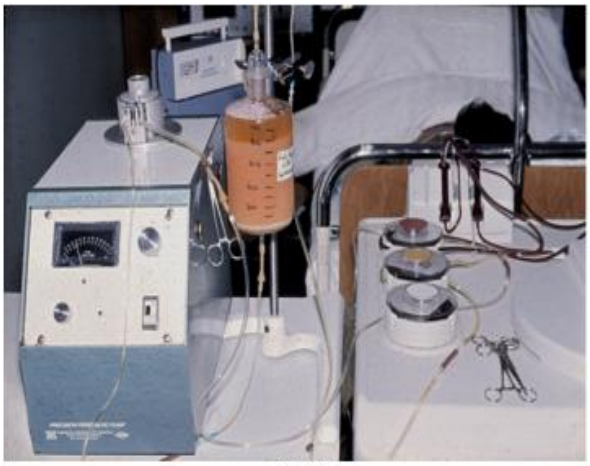
fortunately saved due to the cogency of the biochemical reviewers.
You may e.g. ask how the pig reached the catheter place in the cardiology department. Anaesthesized and hidden in a box of course. The greatest obstacle was overcoming the suspicious specific odor? At this time astronauts circled around the earth for weeks. Life up there was only tolerable because of the deoderant NILIDOR, so also Paula, sleeping and hidden in the box. Coronary angiographies revealed very convincing regression of the atherosclerotic plaques. All this encouraged us to proceed to the
Final goal:
the extracorporeal immuno-adsorption on anti–human low density lipoprotein (LDL) columns in drug resistant familiar hypercholesterolemic patients.

And what about our antiserum donors, the LDL immunized sheep?
I had a class mate, who owned a huge flock of sheep next to the Biocampus Cologne of today. He was an interested shepherd with a great understanding for research. He provided a flock of 12 sheep for our immunization experiments, and took care of our high titer anti LDL sheep, the most valuable we had, and that all without charge!
You may understand our frustration when in 1978, two days before Ramadan they had escaped from the farm. After our search for hours in the suburbs of Cologne was unsuccessful, I made all efforts to protect them from butchering. I ask the broadcasting station WDR for help. They sent a warning message, that the meat of these sheep is highly toxic. Relief

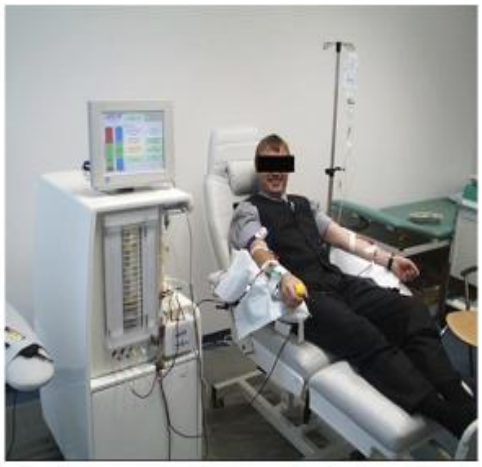
came on Ramadan, when a farmer from a Western suburb of Cologne, Pulheim, called and told me that our sheep were enjoying his clover field.

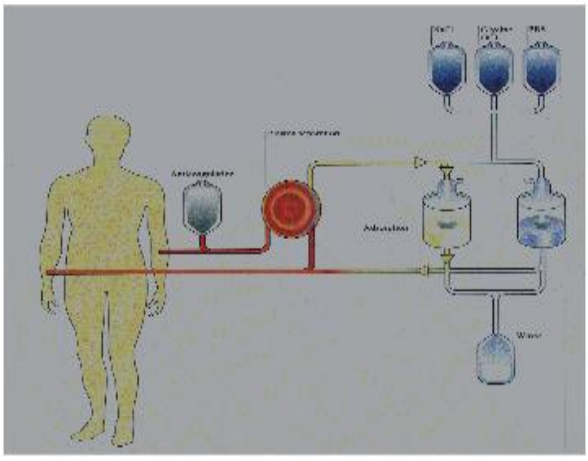
So the project was saved and we produced high quality affinity columns for the first FH patients. Needless to say, that we had taken all security measures possible and standards of today, which are published and I can´t discuss today.

At this time, Volker Greve, another medical Student joined my lab, and I could convince Dr. Helmut Borberg, who was at this time assistant in the Department of Internal Medicine and in charge of the blood cell separation unit, for my project.



1981





Helmut Borberg applied with clinical coolness the first apheresis of a homozygous FHII girl and her brother. Needless

to say, that I was bathed in sweat when I watched the first treatments. The original set up in 1981 is shown here. As of 1982 we improved the system soon after by the two column version allowing alternating loading and desorption. The Version of 2009 is shown below.

LDL depletion kinetics

CLINICAL DATA AND RESULTS

Patient	Total plasma cholesterol levels: Steady state* (mg/dl)	Before treatment† (mg/dl)	After treatment (mg/dl)	Mean between treatments (mg/dl)	Interval between treatments (weeks)	Removed‡: LDL (g)	Cholesterol (g)	Removed cholesterol (%)¶
1	260±30	260	115	203	3	9	4·4	56
		290	130	176	2	9·5	4·8	55
		221	99	169	3	7·5	3·6	55
		238	108			7·5	3·6	55
2	470±15	472	112	223	2	14	7·0	76
		334	153	283	4	11	5·3	54
		412	180	285	3	15	7·3	56
		390	138	247	1	11	5·3	65
		355	113	241	2	11·5	5·7	68
		368	146	251	2	10	5·0	60
		356	133	251	2	12	6·3	63
		369	158	241	2	10	4·7	57
		324	119	207	1	11	5·4	63
		294	95	197	1	9	4·3	68
		298	112	278	5	7·5	3·7	62
		443	164			13	6·4	63
3	500±20	448	171	228	0·5	11	5·3	62
		285	88	183	2	8	3·9	69
		278	103	150	0·5	10	4·9	63
		196	53			6	3·0	74

*Lowest level under medical management. †Immediately before extracorporeal removal of LDL. ‡LDL and cholesterol concentrations recovered from the columns upon regeneration of the immunoadsorbent. ¶Removed cholesterol as a percentage of total cholesterol before treatment.

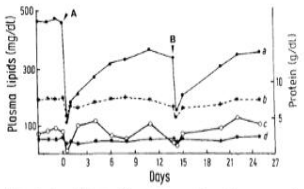


Fig. 2—Immediate and longer-term effects of extracorporeal removal of LDL in patient 2.

A and B are consecutive treatments, separated by 14 days. a=total cholesterol; b=total protein; c=triglycerides; d=HDL-cholesterol.

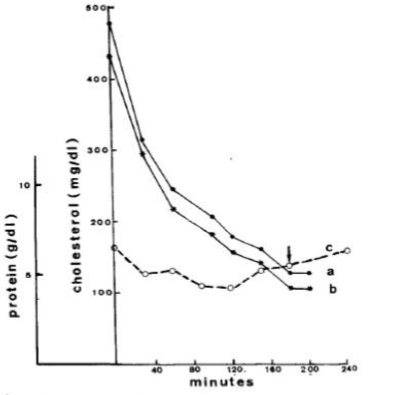
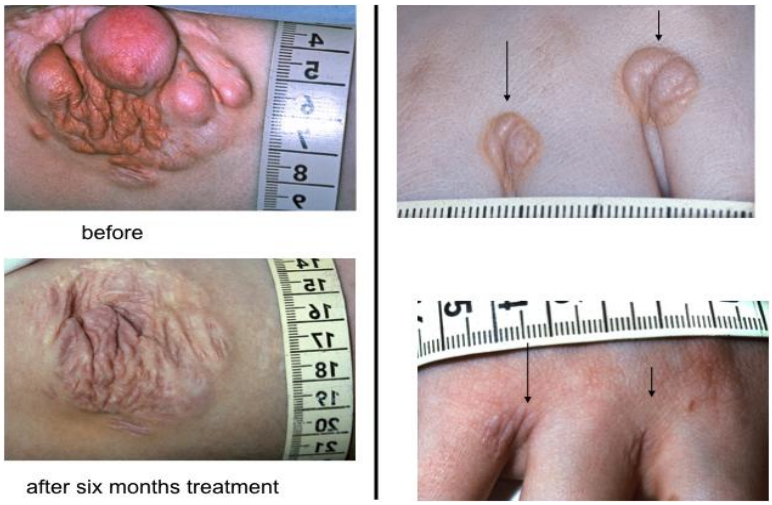


Fig. 1—Time-course of lipid and protein changes during extracorporeal removal of LDL in patient 2.

a=total cholesterol; b=LDL plus VLDL cholesterol; c=plasma protein. An arrow marks termination of treatment. Plasma samples were taken from the plasma line before entering the immunoadsorption column.

As a result of the regular treatment:

Xanthelasmata were massively reduced along with the reduction of atherosclerotic plaques in coronary arteries and aorta, as documented by angiography, performed by young cardiologists, and motivated by the improvement of all CHD symptoms of their patients.



With these first successful treatments I finished this exciting episode in my research and here I stop my short aphoristic excursion into the early history of LDL apheresis. Dr. Schreiner will expand later on the present status and the perspectives of this fascinating therapeutic approach.

This story was written in the beginning by two, later by three actors and our flock of 12 sheep. I enjoyed this time as one of the researcher and as a shepherd.

At the end I would leave you with the rhetoric question, if a complex project as described here briefly, which led to the successful translation of a concept into clinical practice going all the way from basic research to clinical application, could be performed today under the tutelage and torture of a ubiquitous bureaucracy.

Dear Stephan Miltenyi, this recent picture from Teterow shows you as shepherd amidst your flock of sheep. Notice, how closely they surround you. In iconography of all religions and cultures the shepherd is the unifying metaphor for leadership, which demands the most valuable

and desirable virtues. This kind of leadership is a firm basis for the mutual high esteem by all members of the Miltenyi family. I cordially wish that this spirit will further blossom in the future.

New application fields of immunoglobulin G in immuno-adsorption

Hematology	Inhibitors of clotting factors (factor VIII, V, XIII) Inhibitors in hemophilia A und B TTP (Thrombotic thrombocytopenic purpura) Autoimmune hämolytic anemia Bone marrow transplantation (ABO-Incompatibility)
Rheumatology	SLE (systemic Lupus erythematodes), Anti-Phospholipid-Syndrome Rheumatoid Arthritis Collagenoses, Sklerodermia, Dermatomyositis Sjögren-Syndrome Vasculitis Wegener Granulomatosis etc
Nephrology	Myasthenia gravis Guillain-Barré-Syndrome CIDP (Chronisch inflammatorische demyelinating Polyneuropathy) Dysproteinemic Polyneuropathy Multiple Sclerosis Paraneoplastice Syndrome (Lambert-Eaton-Syndrom, Optikusneuropathie, cerebellar degeneration, Myoclonia, anti-Hu-antibodies etc) Goodpasture-Syndrome Nephrotic Syndrome in focal Glomerulosclerose Lupus Nephritis
Cardiology	Dilatative Cardiomyopathy
Gastroenterology	Primary biliary cirrhosis Celiac disease

	Pernicous anemia
Transplantation	Liver and kidney transplantation
Alia	Cryoglobulinemia Insulin-dependent Diabetes mellitus Pemphigus, Urtikaria