History of N-Acetylcysteine

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1.1 N-Acetylcysteine, the Antidote to Acetaminophen Overdose

N-Acetylcysteine (NAC)[1] is a well-known and universally accepted antidote to acetaminophen (APAP; Tylenol, paracetamol) poisoning. Currently in stock in virtually all hospitals and emergency medical facilities throughout the world, NAC has been administered to literally thousands of patients who walked out of their care facilities alive and healthy despite having intentionally or accidentally ingested lethal amounts of APAP.

However, because NAC is readily oxidized to foul-tasting products, it can develop a foul smell that patients commonly find repulsive. I've lost count of the number of physicians who told me "horror stories" about having to convince people who have overdosed on APAP to swallow this life-saving brew.

Nowadays, this scenario is avoided for most APAP overdose patients by giving them NAC intravenously under controlled conditions that allow ready treatment of side effects due to introduction of NAC in this manner. Further, NAC manufacturers have succeeded in producing and packaging NAC under conditions that are fairly successful in minimizing its oxidation, and the oxidation of any impurities, to decrease the foul-tasting contaminants in the product when it has to be given orally. These improvements have greatly simplified and improved the treatment of accidental and intentional APAP overdose, which unfortunately still continue to be a serious public health hazard (Green et al. 2013).

[1] Pronounced either as the homophone for the word "knack" or spelled out as the letters "N-A-C"

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1.2 Unintentional Acetaminophen Overdose

Intentional overdosing with APAP is relatively common, particularly among teenagers. However, such overdosing is only a part of the APAP overdose problem. Several years ago, I was alerted to this when James Andrus, then a young pediatric intensive care physician with whom we did collaborative studies, came into our laboratory looking thoroughly miserable. He told me that he had just admitted the "sweetest little girl," who was rapidly losing liver function for unknown reasons and was likely to die in less than a week unless he could figure out what was going wrong. A few hours later, Jim came back and told me he had decided that although there was no obvious justification, he was going to put the patient on NAC therapy, treating her as if she was suffering from APAP overdose. The therapy started, the child began rapidly began to recover, and was out of the hospital in a week or two.

Jim was delighted, but he really wanted to know what actually caused the liver disease. After a little detective work speaking with the child's family, he ascertained that she was accidentally overdosed with APAP, which is present in most of the over-the-counter (OTC) cold, cough, fever, and headache medications that her family had given her. In essence, she had been suffering from a fairly intense flu-like illness and her parents and grandparents had, over several days, successively given her a large number of APAP-containing OTC medications, individually labeled as useful for reducing one or another of the symptoms of the illness (fever, sore throat, headache, etc.). After several days of this well-intentioned treatment, the child's liver began to fail. She quickly wound up in the hospital with unexplained liver failure, from which Jim's guess about unintentional APAP overdose and his treatment with NAC rescued her.

This is an extreme example. However, the widespread presence of APAP in common OTC medications means that inadvertent overdosing with this drug may be far more common than recognized. Basically, while most physicians are well aware of the dangers of APAP overdose, few recognize how ubiquitous APAP is in OTC medications, how important it is to maintain the *total* APAP exposure within allowable limits, and how low those allowable doses should be. Thus, one important outcome of the publication of this volume should be to sound the call for focus on glutathione (GSH)-depleting medications and diseases and to make pediatricians and physicians in general more aware of both the serious consequences of APAP overdose and the value of treating with NAC where appropriate.

This kind of thinking also raises the question of whether the increase in pediatric APAP use in the United States (US) and several other countries over the last several decades might have contributed to the increased incidence of autism (Tirouvanziam et al. 2012) and other diseases to which "oxidative stress," a corollary of decreased GSH, may contribute (Atkuri et al. 2007). I have seen several studies focused in this way. However, there is certainly good reason to put more effort into examining and documenting the relationship(s) between APAP usage and APAP-caused diseases and conditions.

1.3 Over-the-Counter NAC

Ingested NAC is rapidly deacetylated by first-pass metabolism to yield the amino acid cysteine, which accumulates in the liver as such and is also incorporated into a key tripeptide, GSH (γ-glutamylcysteinylglycine). GSH, cysteine, and methionine (which can be converted to cysteine) then accumulate in the liver and are doled out to the rest of the body as needed for protein synthesis and for maintenance of GSH levels in cells. Thus, the Food and Drug Administration (FDA) classifies NAC as a nutraceutical, even though it is clearly used for medicinal purposes.

Much of the NAC sold in health food and similar food or drug stores today is packaged in containers that are not designed to protect NAC from air oxidation, and hence deliver increasingly more di-NAC as packages age, particularly when opened. I know of two companies, Zambon Pharma (Italy) and BioAdvantex (Canada and US) that currently produce and package NAC in ways to minimize oxidation. Zambon offers NAC as Fluimucil tablets, packaged in relatively small tubes that minimize NAC exposure to air; BioAdvantex offers NAC as PharmaNAC, effervescent "fizzy tabs" that readily dissolve in water, juice, or soda and are individually packaged in sealed foil packets to minimize exposure to air. Both companies have supplied NAC and placebo for clinical trials in our laboratory and elsewhere.[2]

To my knowledge, despite the growing use of NAC in medical practice discussed in this volume, neither these nor any other NAC-producing company has yet mounted a serious NAC-APAP production effort aimed at providing a safer APAP product.

1.4 NAC-Acetaminophen: Formulate the Antidote Along with the Drug?

The FDA, of course, is well aware that APAP overdose is a public health hazard. On at least two occasions, they have decreed a reduction in the allowable APAP dosage in OTC medications. This has certainly been helpful, but unintentional overdose is still a problem.

Of course, one could ask, since NAC has been found to be a safe antidote to APAP toxicity, "why not prevent acetaminophen overdose problems altogether by mandating that each acetaminophen tablet be formulated with enough NAC to prevent the ingested acetaminophen from significantly decreasing patient glutathione levels?" I'm not sure whether this issue has been discussed by any of the major APAP producers, who perhaps lack an incentive to point out that their APAP products may be toxic at doses that fall far short of typical toxic APAP overdosing.

[2] Disclaimer: In accord with our university policy, we have cooperated with BioAdvantex in the development of several patents for NAC use in HIV and other diseases and have worked on clinical trials with both Zambon and BioAdvantex. Some of these patents have been issued; none have as yet secured FDA approval for clinical (or other) uses. Other groups conducting studies discussed in this volume have worked, I believe, with other NAC sources

The failure to field (or mandate) marketing of a NAC-APAP product may also be explained by a relatively short shelf life of *reduced* NAC, which, unless properly sequestered, is rapidly oxidized in air to "di-NAC" (NAC-S-S-NAC). Di-NAC has been reported to be highly pro-inflammatory, and hence its presence, even at low levels in unprotected NAC formulations, could subvert the anti-inflammatory activity of the NAC monomer. Unfortunately, the larger medical community has not embraced this understanding of NAC. For example, in a recent very large clinical trial using NAC, this aspect of protecting the product from oxidation was not addressed (Weisbord et al. 2018).

The failure to create and market a NAC-APAP product could be due to the bad sulfurous smell/taste that accumulates when NAC is not properly sequestered and can often be detected when typical off-the-shelf NAC packages are opened. Any or all of these reasons could account for reluctance on the part of APAP producers to coformulate their product with NAC.

Going in the other direction, I raised this coformulation idea with a small company (BioAdvantex.com) that produces and safely packages NAC to protect it from becoming oxidized. They package the NAC in individually sealed foil packets that minimize NAC exposure to air and thereby prevent significant NAC oxidation and/or generation of foul smells or tastes. The company has sold NAC in such protective packaging for many years and is the mainstay of many people who depend on NAC for medical reasons. However, although this company recognizes the importance of fielding a combined NAC-APAP OTC medication, they have not yet been able to develop the resources to mount the extensive effort required to generate and get approval for this NAC-APAP[3] product. Thus, at present, there is no viable source for a properly protected product designed to provide normal APAP doses along with sufficient NAC to prevent the toxic effects of the ingested APAP.

1.5 How Did We, Two Basic Scientists, Get Involved with Considering the Clinical Uses of NAC?

Having studied at Caltech in the mid-1950s, when biochemical genetics was king, Len (my late husband) and I started out being more familiar with GSH than most of our current medical and genetics colleagues. Len's thesis work (and my "tagalong") brought us into contact with cytochromes, mitochondria, respiratory pathways, and the like, and his postdoctoral work (and again, my tagalong work) with Jacques Monod in Paris studying enzyme (β-galactosidase) induction in *Escherichia coli* taught us more basic biochemistry, cell physiology, and genetics.

When we moved to the National Institutes of Health (NIH) so Len could complete his required "military" service, I continued with bacterial genetics/physiology work

[3] In accord with our university policy, we have been joined by this company (BioAdvantex) in the development of several patents for NAC use in HIV and other diseases. However, neither we nor BioAdvantex have secured FDA approval for any clinical (or other) uses.

in Bruce Ames laboratory. Len, however, followed his "dream" and moved to Harry Eagle's laboratory, where mammalian cells were being put into culture for the first time. Joining the effort to craft culture media for these cells, Len found that the cultured cells had a curious need for a metabolite (pyruvate) that they were quite capable of making. Understanding the biochemistry surrounding this "oddity" and other such findings in Eagle's laboratory gave us a solid background for our later explorations of the interplay between biochemical and genetic mechanisms (including redox) and the survival, growth, genetics, and function of mammalian cells. Thus, by the time Len was appointed (and I tagged along) to the faculty of Joshua Lederberg's newly founded Genetics Department at Stanford, we were well primed to begin the mammalian genetics and cell biology studies that have occupied us ever since.

Len initially focused on biochemical and drug sensitivity markers expressed by mammalian cell lines. However, soon after we arrived at Stanford, Lederberg lodged us in a laboratory next door to where Gus Nossal and Olli Makela were engaged in proving that "one cell makes only one antibody." This idea is at the heart of the Lederberg-Burnet theory of antigen-based selection of cells. At this time, medical science believed that all antibodies had the same structure, which then folded in different ways to combine with different antigens.

The work going on in the lab next door was too exciting to just pass by. Len suggested that I learn how to immunize mice and measure antibodies. I did, and within a short time, we were both seduced into in vivo immunology and mouse immunogenetic studies. In the ensuing years, this led to our building the fluorescence-activated cell sorter (FACS), making monoclonal antibodies to identify, sort, and test immunologically relevant mammalian cells, and to using these antibodies and the cell sorter to clone CD5, CD8, and other immunologically relevant mammalian genes.

Relevant to the redox focus of this article, Len and his fellows developed a FACS-detectable reporter gene assay in which they introduced *Escherichia coli* β-galactosidase gene (lacZ) under the control of various genetic regulatory elements (e.g., NF-κB) into cell lines. They then used FACS to measure lacZ cleavage of a fluorogenic substrate in individual cells as an index of promoter efficacy, enhancer activity, trans-acting factors, etc. Thus, we were well prepared to determine the sensitivity of the promoter elements to various stimuli and/or stimulatory conditions, including oxidative stress. Indeed, we later showed, the expression β-galactosidase (lacZ) reporter gene under the control of NF-κB in these various promoter constructs increases in cell lines grown under oxidative stress and decreases when NAC was added to the cultures to reduce oxidative stress (Nolan et al. 1988).

1.6 Growing Cells at Physiological Oxygen Levels

In our early studies we were not equipped to alter our incubator oxygen levels. However, modern ("Tri-Gas") incubators that are now commonly available have the ability to mix and maintain the concentrations of three gases (O_2, CO_2, and nitrogen). Nevertheless, most incubators today are still run with only two gases, 5% CO_2

and air, which results in continuously exposing cells to oxygen levels of about 20%. Since, except for skin and other tissues exposed to air, mammalian cells live at oxygen levels considerably below this (roughly 2–12% oxygen), much of the work with mammalian cells and cell lines has been done with cells growing under significant oxidative stress.

Thus, not surprisingly, when cells are grown in incubators maintained at physiological oxygen levels (5–10% O_2 in a 5% CO_2 "Tri-Gas" incubator), their responses differ in key ways from that are cells grown in incubators maintained at room air oxygen levels. For example, sensitivity to apoptosis induction by HIV-Tat, a widely studied apoptosis inducer, is markedly decreased in cells grown at 5% O_2 versus cells grown at typical incubator oxygen levels (20%). These findings underscore the importance of taking incubator oxygen levels into account when interpreting data from cell culture studies, which today unfortunately are largely conducted at room air.

Adding NAC to cells cultured at 20% O_2 can decrease the oxidative stress, as our early findings with the human immunodeficiency virus (HIV) promoter demonstrated. However, preventing the stress in the first place by lowering the incubator oxygen levels that approximate those the cultured cells encounter in vivo (generally 5–10%) is likely better in the long run. In fact, in vivo *veritas* is probably still a good rule to follow, if experimental goals permit. Keeping incubator oxygen levels in an appropriate range for the cells being cultured is likely to provide findings more suitable for predicting in vivo behavior of the cultured cells.

1.7 NAC and HIV

Some time after we had developed these constructs discussed above, Len and I chanced to hear a seminar by the National Institute of Allergy and Infectious Disease (NIAID) director Anthony Fauci, who reported work done with Mary Anderson and Alton Meister demonstrating NAC's interference with HIV replication in cell lines. Since NF-κB was already known to be an important regulatory element in the HIV promoter, we were immediately energized to use our NF-κB lacZ reporter line to determine whether NAC would decrease lacZ expression upregulated in the cell line under oxidative stress. Indeed, as predicted, we found that adding NAC to the culture downregulated the activity of the NF-κB transcription factor in several reporter constructs, including those in which the HIV promoter controlled lacZ expression.

These findings suggested that ingesting NAC might downregulate HIV expression in HIV-infected individuals. And so began our long and still continuing romance with NAC as a supplementary source of dietary cysteine necessary not only for protein synthesis but also for synthesis and maintenance of reduced intracellular GSH, the primary intracellular antioxidant, a key regulator of intracellular redox status and, in vitro at least, a key regulator of the HIV activity (Staal et al. 1990).

On hearing the above findings, Fauci and his group rapidly decided to run a quick (2-week) trial to determine whether NAC would be an antiretroviral drug and hence

would decrease viral load very rapidly. It did not. Further, although administered at a high dose, NAC proved to be minimally detectable in circulation shortly after dosing. This failure to detect NAC in the circulation is not surprising, since orally administered NAC is rapidly metabolized to cysteine once it leaves the digestive tract and enters the liver. However, together with the failure to rapidly achieve the desired antiretroviral effect, this apparent lack of NAC's ability to "get in" was sufficient for Fauci's group to rapidly cross NAC of the list of potential therapeutics in HIV infection. This decision paid off, since it ultimately led to the discovery of the antiretroviral azidothymidine (AZT), which of course went on to become the first of a series of progressively more effective and less toxic therapeutics for treating HIV infection.

Of course, orally administered NAC "gets in" since, as indicated above, its administration is the standard of care for treating toxic APAP overdose. Therefore, although NAC failed as a rapid antiretroviral, we mustered our resources and established a small, short-term (8-week) placebo-controlled double-blind study to determine what benefit, if any, NAC therapy could bring to HIV-infected patients.

Results from this trial were largely eclipsed by the spectacular results obtained with AZT, which were published about the time we were ready to publish our findings. However, our findings established that HIV-infection progressively decreases intracellular GSH levels, that subjects with CD4 T-cell counts below 200 had the lowest GSH levels, and that NAC restores the diminished GSH by the end of the 8-week trial period (Herzenberg et al. 1997; Herzenberg et al. 1998; De Rosa et al. 2000).

We were not confident, or arrogant, enough to set survival as an endpoint for this trial. However, when we surveyed the survival of the subjects 1–2 years after the trial, we found that none of the participants with T-cell counts above 200 had died, that a sizable number with CD4 T-cell counts below 200 had died, and that within this group, the survival of subjects who received NAC was significantly greater than those who had received the placebo. Kaplan-Meier analysis added that even the NAC-taking subjects who died had survived significantly longer than those who were given placebo.

Our findings with this study were eclipsed by spectacular findings obtained with AZT treatment, which were published before or around the same time. As a result, AZT treatment was prescribed for essentially all HIV patients whose survival was at risk. Therefore, there was no justification for continuing our NAC-HIV studies except as adjunct therapy, for which were unlikely to get support since the HIV research community largely still believed that orally administered NAC "does not get in."

Nevertheless, we were encouraged to go on with our NAC-HIV and other NAC-redox studies based on the results from our in vitro reporter gene studies (Herzenberg et al. 1997; Herzenberg et al. 1998; De Rosa et al. 2000), our clinical trial testing NAC in HIV-infected subjects (Herzenberg et al. 1997; Herzenberg et al. 1998; De Rosa et al. 2000), and a series of positive anecdotal results reported by HIV-infected individuals who took NAC. Ultimately, our work and our interests broadened to include findings with NAC treatment in other diseases,

including our collaborative clinical studies showing positive effects for NAC treatment in cystic fibrosis (CF) (Tirouvanziam et al. 2006) and autism (Hardan et al. 2012; Tirouvanziam et al. 2012).

1.8 NAC Treatment in Disease

We had started the above clinical HIV studies in accord with a suggestion by a German T-cell biologist, Wulf Droge (now deceased), who had conducted a long series of in vitro studies characterizing the amino acid and other requirements for T-cell growth and function in vitro. On hearing about this work, a pharmacist who lived next door to Wulf asked him whether he could suggest anything that might help his adult HIV-infected son, whose T-cell counts had already diminished markedly as had his ability to help his father in the pharmacy. Wulf reasoned that some help might be gotten by increasing the dietary intake of certain amino acids that he found were necessary for T-cell growth in vitro, naming the sulfur-containing amino acid cysteine as one of these.

The pharmacist, knowing that oral NAC is administered to counter cysteine and GSH loss in APAP toxicity, responded by questioning whether administration of the medicinal grade NAC he had in his pharmacy might be useful in restoring his son's health. Wulf was not sure, but he agreed that since NAC is not known to be toxic, the pharmacist should try administering it to his son. Surprisingly, within a short time of initiating this NAC treatment, the pharmacist's son regained strength, got back to work, and remained relatively healthy for some years until other aspects of HIV disease caught up with him.

Seeing this "miracle," Wulf decided to tell this story in an open letter to 100 immunologists, with the hope that someone would be encouraged to investigate NAC as a potential therapeutic for HIV disease. So far as we know, only one laboratory (ours) took this idea seriously at the time. Working, as we were, only a short distance from San Francisco, we were seeing and hearing close at hand the mounting numbers of HIV deaths in the city. Anything that could help should be considered.

Bringing this plague even closer to home, we had recently learned that a close friend, a gay man working as central engineer/investigator on our FACS development staff, was infected with HIV. Therefore, we were very attuned to Wulf's message suggesting that a nontoxic treatment, NAC, might comfortably contribute to prolonging life in HIV infection and gratefully accepted Wulf's offer to send a "care package" with enough NAC for several months' treatment for our friend and his partner.

At the time, our friend was helping his domestic partner through the last stages of an opportunistic infection and did not want to try anything new for himself or his partner. However, at his suggestion, we gave the NAC to another gay couple, mutual friends also moving toward end-stage acquired immune deficiency syndrome (AIDS). We were surprised and very pleased to see that after a few weeks on NAC (in addition to their medically approved treatment), both friends felt healthier and

resumed folk dancing and other activities that their disease had forced them to curtail. When last heard, they had set out on a world tour to "see the places they thought they would never get to see."

Our staff member continued to care for his partner through a sad and immensely painful passing, and was then cut adrift to face his own likely demise. Being quite knowledgeable about the horrendous effects of the then early HIV drugs, he decided not to use any at all. We understood and said we would support him and help him face the potentially painful consequences of this nonstandard response. However, we asked him to take NAC regularly and continued to monitor his T-cell counts as he went along (he had 300–400 CD4 T-cells at the time). His physician was not so sanguine about his choice, but could do nothing to convince him to do otherwise.

Once our friend started taking NAC, his CD4 T-cell counts stabilized in the 300–400 range and only fell to around 200 several years later. By that time, less toxic HIV drugs were available and he decided to take these. Currently, having shifted to better and better drugs in the intervening years, his CD4 T-cell counts are in the very desirable 600 range, and he has ridden his Harley to Canada and Alaska multiple times over the last few years (at this writing he is in Canada enjoying another such ride). Importantly, those of us using FACS have all benefited from his continued good health, since over the years, he has developed FACS/Desk and other innovative mathematics and software that collectively underlie the current FlowJo FACS/CyTOF data analysis program (FlowJo.com) and his/our own CytoGenie software, which provides innovative, statistically based software for FACS/CyTOF data analysis and visualization.

Anecdotal stories like this of course mean very little. Clearly, we would love to see whether a properly controlled trial for NAC as adjunct therapy for HIV would confirm our observations. However, the likelihood of such a trial happening in the near future seems slim indeed in the current world. Therefore, since NAC is not toxic, we content ourselves with recommending that HIV-infected individual supplement a good diet and medication regime with NAC, properly packaged to prevent it from being oxidized while "waiting on the shelf" to be ingested.

Nevertheless, we still continued to measure GSH levels in T-cells from HIV-infected people and to correlate these levels with disease parameters. We also developed a more facile test for determining GSH levels in HIV-infected patients and correlating the measured levels with survival. Using this test, we found again that GSH levels decrease as patient conditions deteriorate.

1.9 Contrast Nephropathy

Several years ago, our colleagues and us attempted to put together a comprehensive review of NAC uses in the clinic. It proved to be a gargantuan task, well beyond what we could manage, and the data available at the time was far too sparse**.** Nevertheless, we did learn some useful things, particularly about what has now become the fairly routine use of NAC to prevent the development of contrast dye-induced nephropathy in patients scheduled for various radiological procedures.

A survey of the literature available at the time (ca 2010) revealed a striking dichotomy in the reported results, some studies showing clear success in the NAC versus placebo groups, while others showed no differences at all. Broken down further into the study locale, we surprisingly found that studies conducted in Europe were far more likely to show that NAC is successful in preventing nephropathy than studies conducted in the US.

We never arrived at a satisfactory explanation for this observed difference. The patient groups and the trial protocols seemed comparable in the two locales. However, there was likely to have been key differences in the source of the NAC used in the two studies. That is, NAC is commonly used in Europe to treat asthma and a variety of other conditions and hence is packaged and sold under stringent conditions that mitigate against inclusion of oxidized NAC in the product. In contrast, in the US, relatively palatable NAC is commonly sold in health food and other stores that pay little attention to whether care is taken to prevent oxidation of the NAC, either before or after packaging. Since oxidized NAC can be expected to act antithetically to reduced NAC, the differences in study results could well be explained by differences in the oxidative state of the NAC at the time of treatment. Because of this, we used NAC that was properly packaged according to European standards in our studies.

This explanation occurred to us as we collated the data for the contrast nephropathy section of the review we were trying to write. However, although we sent letters and made phone calls requesting specification of the source of the NAC used in the various studies, and although we followed up on most of these requests, we did not receive useful answers from most of the authors of the published studies. Thus, while we believe the source of the NAC and its likelihood of being oxidized may explain the differences in outcomes in these studies, we have no direct evidence to substantiate this claim.

Nowadays, despite the earlier conflicting results, physicians in the US commonly recommend taking NAC some hours prior to exposure to contrast dyes. Fortunately, because they tend to actually prescribe the NAC, it will usually be obtained from a reputable pharmacy that will fill the prescription with pharmaceutical grade NAC used also to treat APAP overdose. Thus, the prescribed NAC may taste terrible, but will likely be safe and efficacious. In Europe, in contrast, NAC is routinely produced under European standards and packaged to prevent or at least minimize oxidation. Thus, it is available from reputable companies (e.g., Zambon, Inc.). In the US, BioAdvantex sells European-produced NAC, mainly via mail or web order.

1.10 Cystic Fibrosis

Some time ago, Dr. Rabindra (Rabin) Tirouvanziam, working in our laboratory, decided to determine whether NAC would be useful for treating cystic fibrosis (CF). Teaming up with Drs. Richard (Rick) Moss and Carol Conrad from the Stanford Medical School CF Clinic, Rabin initially organized a Phase 1 trial that was

supported by the US FDA orphan drug unit. Using NAC supplied by BioAdvantex, Rabin and Carol obtained positive Phase I results and embarked on organizing a Phase 2 trial testing NAC efficacy in treating CF.

Here, the story becomes more complex. Carol Conrad, who leads the CF clinic at the Stanford Medical School, became the principal investigator for the planned multicenter trial, with Rick Moss as consultant. Carol and Rabin went to visit the CF foundation to request help and advice in setting the trial guidelines and determining the trial endpoint(s). Richard Moss, who has led the Stanford clinical effort in CF for years, and I (a non-clinician) were pleased that our younger colleagues were "carrying the ball" and left them to it. We did not visit the foundation with them.

At the end of the day, Carol and Rabin sifted the advice they received from the foundation and established a defined magnitude change in sputum human neutrophil elastase (HNE) activity as the primary endpoint. As secondary endpoints, they specified a change in forced expiratory volume in 1 s (FEV1) and other clinical lung function measures that are commonly considered unlikely to change significantly during trials. They also specified the safety and tolerability of NAC and the potential of NAC to promote pulmonary hypertension in subjects with CF as additional endpoints.

The results from this trial proved to be quite surprising. Although HNE is considered to be a good CF clinical endpoint, there was no significant difference between the NAC-treated and placebo control groups. On the other hand, FEV1 increased significantly in the treated group but stayed constant or fell somewhat in the placebo control (Conrad et al. 2015). Since an increase in FEV1 is extremely rare in CF patients, this latter finding suggests that NAC is indeed beneficial for the treated patients.

Ideally, this study should be repeated with FEV1 as the primary endpoint. However, CF is classified by the FDA as an orphan disease, meaning there are too few patients to be able to conduct and repeat trials. In essence, the CF patient pool is probably not large enough to enable recruitment for a second trial, particularly since many CF patients heard about our results and are now "self-dosing" with NAC, which is available OTC in the precise individually sealed packaging used for the trial.

We plan to query the FDA to determine how to move forward within the confines of this result. However, the likelihood is that NAC treatment in CF will be left up to individual physicians and their patients, who will have to find and purchase properly packaged NAC and find the funds to support NAC treatment in the absence of FDA approval and hence the consequent lack of insurance funding for the treatment.

1.11 Autism

One day, as our NAC studies proceeded, a senior administrator in the Stanford Immunology Program came by and asked me whether I thought NAC treatment could be useful in autism. Knowing that she had two autistic children, I didn't

simply brush off the query but discussed the issue with her. She told me about another Stanford Medical School professor who had a child who was much less affected than her boys and suggested he might be interested in trying NAC with his son. Since NAC is not known to be toxic, I suggested that I could procure European-style NAC (as it has come to be known) if the faculty member she spoke about was interested. She then invited this professor to meet me to discuss the issue.

When we met, I outlined what I knew about NAC and said that I would be willing to procure the NAC if the NAC treatment were done under the eyes of an autism specialist. This was readily arranged since the child's current physician was just such a specialist. Over time, the NAC treatment appeared to have beneficial effects for the child, leading Dr. Antonio Hardan in the Stanford Pediatrics autism group to establish a serious placebo-controlled, double-blind clinical trial testing NAC for efficacy in autism, which proved successful in that the treatment group showing improvement in key widely used assays of behavioral function (Hardan et al. 2012).

The trial was successful for me in other ways, too, in that Dr. Hardan introduced me to Dr. Michael Berk, the coeditor of this volume, who, together with his coeditor Dr. Richard Frye, invited me to wander through my laboratory's interest in NAC and redox, and to lightly summarize our path as an introduction to this wonderful volume.

1.12 Summary

In the sections above, I have outlined some basic and some clinically related findings from the NAC studies we have conducted in our laboratory or encountered in our travels. The chapters that follow in this book tell a surprising and far broader story of NAC's uses than my husband and I would have ever thought possible when we first began thinking about NAC and NF-κB years ago. Actually, though, we all should be surprised—surprised that it took us, as a scientific and medical community, so long to recognize the importance of the complex relationship between the air we breathe and the life we lead. Just because we don't see air and the oxygen it contains does not mean we should forget that it is there. This book reminds us of this, and brings home to us how far we have come, and how much farther we have to go to understand the processes that together serve the living organism. At least we, a research and medical community, have now made a strong beginning.

References

Atkuri KR, Mantovani JJ, Herzenberg LA, Herzenberg LA (2007) N-acetylcysteine-a safe antidote for cysteine/glutathione deficiency. Curr Opin Pharmacol 7(4):355–359

Conrad C, Lymp J, Thompson V, Dunn C, Davies Z, Chatfield B, Nichols D, Clancy J, Vender R, Egan ME, Quittell L, Michelson P, Antony V, Spahr J, Rubenstein RC, Moss RB, Herzenberg LA, Goss CH, Tirouvanziam R (2015) Long-term treatment with oral N-acetylcysteine: affects lung function but not sputum inflammation in cystic fibrosis subjects. A phase II randomized placebo-controlled trial. J Cyst Fibros 14(2):219–227. https://doi.org/10.1016/j.jcf.2014.08.008

De Rosa SC, Zaretsky MD, Dubs JG, Roederer M, Anderson M, Green A, Mitra D, Watanabe N, Nakamura H, Tjioe I, Deresinski SC, Moore WA, Ela SW, Parks D, Herzenberg LA, Herzenberg LA (2000) N-acetylcysteine (NAC) replenishes glutathione in HIV infection. Eur J Clin Investig 30(10):841–856

Green JL, Heard KJ, Reynolds KM, Albert D (2013) Oral and intravenous acetylcysteine for treatment of acetaminophen toxicity: a systematic review and meta-analysis. West J Emerg Med 14(3):218–226. https://doi.org/10.5811/westjem.2012.4.6885

Hardan AY, Fung LK, Libove RA, Obukhanych TV, Nair S, Herzenberg LA, Frazier TW, Tirouvanziam R (2012) A randomized controlled pilot trial of oral N-acetylcysteine in children with autism. Biol Psychiatry 71(11):956–961. https://doi.org/10.1016/j.biopsych.2012.01.014

Herzenberg LA, De Rosa SC, Dubs JG, Roederer M, Anderson MT, Ela SW, Deresinski SC (1997) Glutathione deficiency is associated with impaired survival in HIV disease. Proc Natl Acad Sci U S A 94(5):1967–1972

Herzenberg LA, De Rosa SC, Herzenberg LA (1998) Low glutathione levels in CD4 T cells predict poor survival in AIDS; N-acetylcysteine may improve survival. In: Monatagnier LOR, Pasquier C (eds) Oxidative stress in cancer, AIDS and neurodegenerative diseases, vol 1. Marcel Dekker, Inc., Stanford, pp 379–387

Nolan GP, Fiering S, Nicolas JF, Herzenberg LA (1988) Fluorescence-activated cell analysis and sorting of viable mammalian cells based on beta-D-galactosidase activity after transduction of Escherichia coli lacZ. Proc Natl Acad Sci U S A 85(8):2603–2607

Staal FJ, Roederer M, Herzenberg LA, Herzenberg LA (1990) Intracellular thiols regulate activation of nuclear factor kappa B and transcription of human immunodeficiency virus. Proc Natl Acad Sci U S A 87(24):9943–9947

Tirouvanziam R, Conrad CK, Bottiglieri T, Herzenberg LA, Moss RB (2006) High-dose oral N-acetylcysteine, a glutathione prodrug, modulates inflammation in cystic fibrosis. Proc Natl Acad Sci U S A 103(12):4628–4633. https://doi.org/10.1073/pnas.0511304103

Tirouvanziam R, Obukhanych TV, Laval J, Aronov PA, Libove R, Banerjee AG, Parker KJ, O'Hara R, Herzenberg LA, Herzenberg LA, Hardan AY (2012) Distinct plasma profile of polar neutral amino acids, leucine, and glutamate in children with autism Spectrum disorders. J Autism Dev Disord 42(5):827–836. https://doi.org/10.1007/s10803-011-1314-x

Weisbord SD, Gallagher M, Jneid H, Garcia S, Cass A, Thwin S-S, Conner TA, Chertow GM, Bhatt DL, Shunk K, Parikh CR, McFalls EO, Brophy M, Ferguson R, Hongsheng W, Androsenko M, Myles J, Kaufman J, Palevsky PM (2018) Outcomes after angiography with sodium bicarbonate and acetylcysteine. N Engl J Med 378(7):603–614