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A Vaccination Technique to Combat Presently Untreatable Chronic Ailments

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Humans have enjoyed large-scale protection against many infectious and contagious diseases since 1796, when Edward Jenner first introduced a vaccination against smallpox by an active immunization technique. Vaccination has proved itself to be the most successful solution for preventing the occurrence of many infectious diseases that previously caused serious illnesses, post-recovery ailments, and even death (*e.g.*, smallpox, diphtheria).

Another vaccination technique—passive immunization—was introduced by Emil von Behring in 1891 to protect against diphtheria, and later on against tetanus. Passive immunization requires the preparation of suitable antibodies (Abs) against an antigenic component (*e.g.*, in diphtheria, against the disease-causing diphtheria toxin). Intravenous immunoglobulin (IV Ig) is also used for the prevention and treatment of certain disorders.[1-5] Passive immunization and IV Ig use necessitates the injection of relatively large volumes (and high concentrations) of effective Abs against the offending antigen (Ag) in order for them to be effective. Both active and passive vaccination techniques are utilized to combat exogenous Ag-caused diseases, though active immunization is our primary mode of defense against them.

To date, there has been no effective vaccination technique to deal with endogenous Ag-caused mishaps. There have been attempts to use both active and passive vaccination techniques to induce or introduce corrective immune responses in both autoimmune disorders and cancer, but so far only marginally significant results have been obtained.[1,5-11]

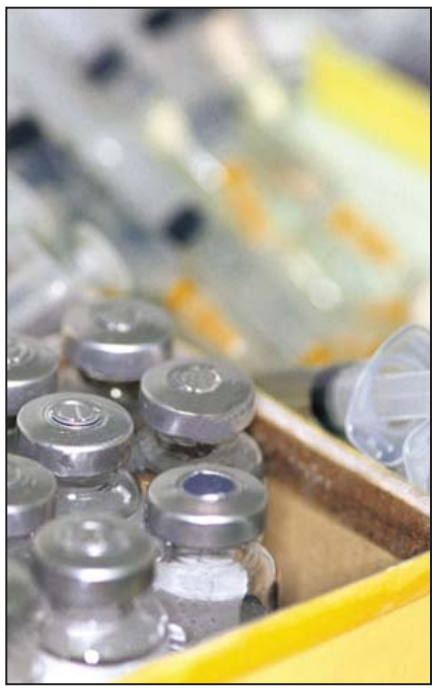
The reason why endogenous Ag-derived diseases have so far thwarted efforts at prevention and treatment is that we have not clearly understood the workings of the body's autoimmune network. Even today, most scientists consider autoimmunity to be a harmful immune response. It often results in serious disorders, producing irreversible functional and morphological changes in the affected organ, along with the associated clinical signs and symptoms.[12]

We believe that the autoimmune system actually serves a wide range of beneficial functions in maintaining tolerance to self. In fact, this arm of the immune system, perhaps along with regulatory cells such as T cells, is responsible for protecting the individual from autogenous disorders (*i.e.*, disorders caused by endogenous autoantigen [AAg] presentation abnormalities[13]) such as autoimmune disorders and cancer.

In order to adequately vaccinate against endogenous Ag-derived disorders, it is necessary to fully understand the workings of the autoimmune system—the good and the bad, the beneficial and harmful aspects (Figure 1). We must also know how to introduce the endogenous Ag to the cells of the immune system in order to down-regulate (in an autoimmune disease) or upregulate (in cancer) immune responses specifically, and without causing side-effects (Figures 2 and 3).[14,15] The task of initiating Ag-specific immune responses is not easy because of the complex nature of the autoimmune network.

Additional factors that complicate or prevent specific treatment of autoimmune disorders include the following:

• Autoimmune disorders can be initiated by numerous etiological factors,



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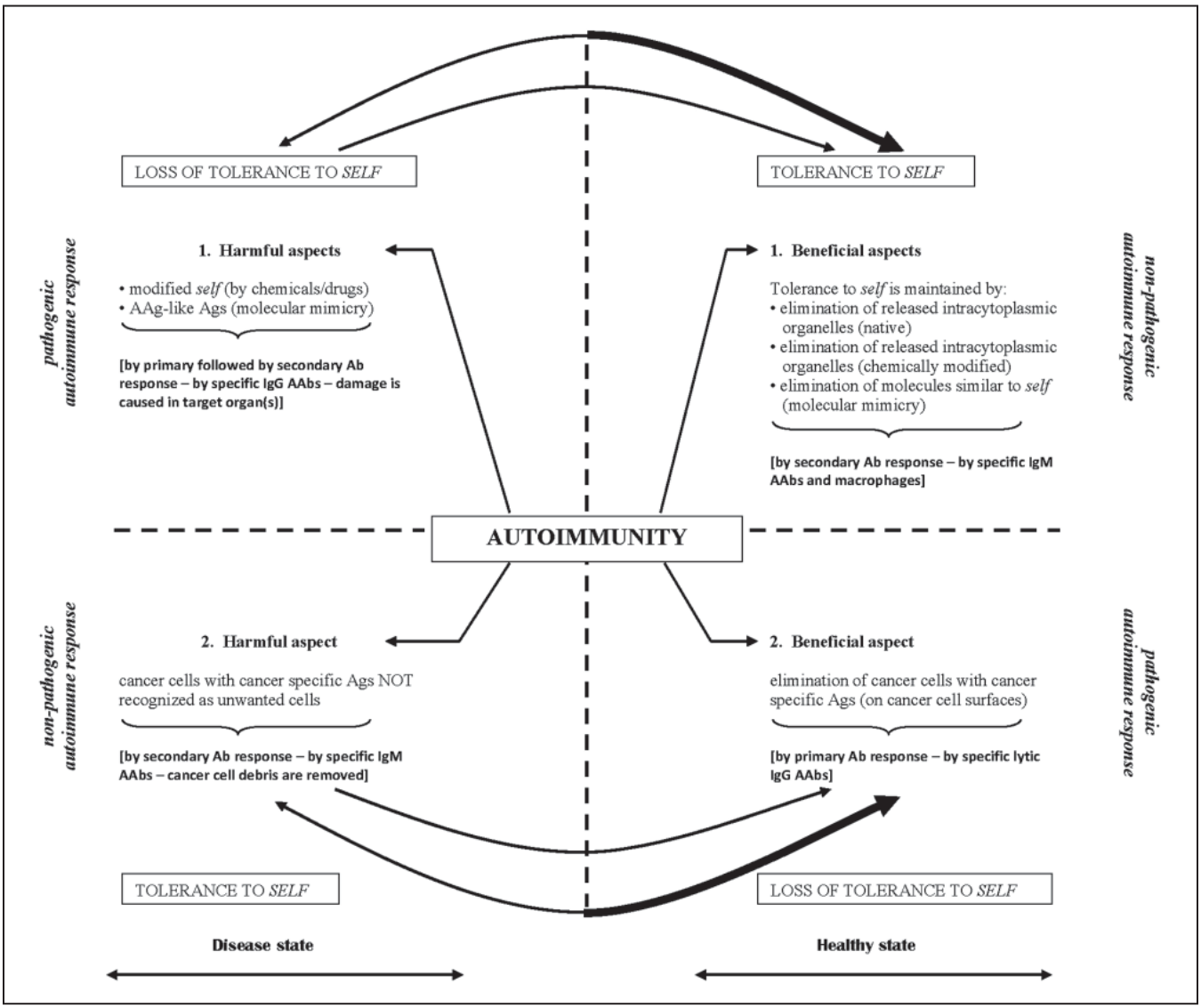

Figure 1. Beneficial and harmful aspects of autoimmunity. The diagram depicts four autoimmune events that can be beneficial or harmful to the individual. The aim is to regain tolerance to self as quickly as possible by specific, naturally occurring immune events (*e.g.*, by accelerated removal of intracytoplasmic components in an autoimmune disease); however, if it is not achieved by the immune system on its own, then it can be accomplished by the appropriate application of the MVT.

and most often long before the diseases are diagnosed.

• Disease initiating and maintaining etiologies in many instances are not known.

• The pathogenesis of many autoimmune disorders is not fully understood.

• The role of pathogenic and non-pathogenic autoimmune responses during disease is not understood.

• How to achieve Ag-specific prevention and downregulation of autoimmune diseases by immunological means is unknown (except in a few cases where prevention has been achieved).

Not fully comprehending the etiologies and pathogenesis of most experimental and human autoimmune disorders prevents us from applying specific treatments. As a result, immunosuppressive agents are still used to treat patients with autoimmune disorders.[16,17]

Prevention of autoimmune disorders has been successfully accomplished in some cases by Ag-specific treatment protocols, using soluble target tissue Ags administered by various routes. For example, in some cases animals that received native, soluble tissue Ags prior to the induction of a disease by usual techniques developed no autoimmune disorder, or a milder form of the disease.[18-22] However, when the same soluble Ags were administered during an established autoimmune disease, no beneficial advantage was observed; and in a few cases, accelerated responses were noted.[9,40,42]

We have worked out a new vaccination method originating in a slowly progressive Heymann nephritis (SPHN) autoimmune kidney disease model[12,23] that works for both prevention and treatment. We consider it to be the

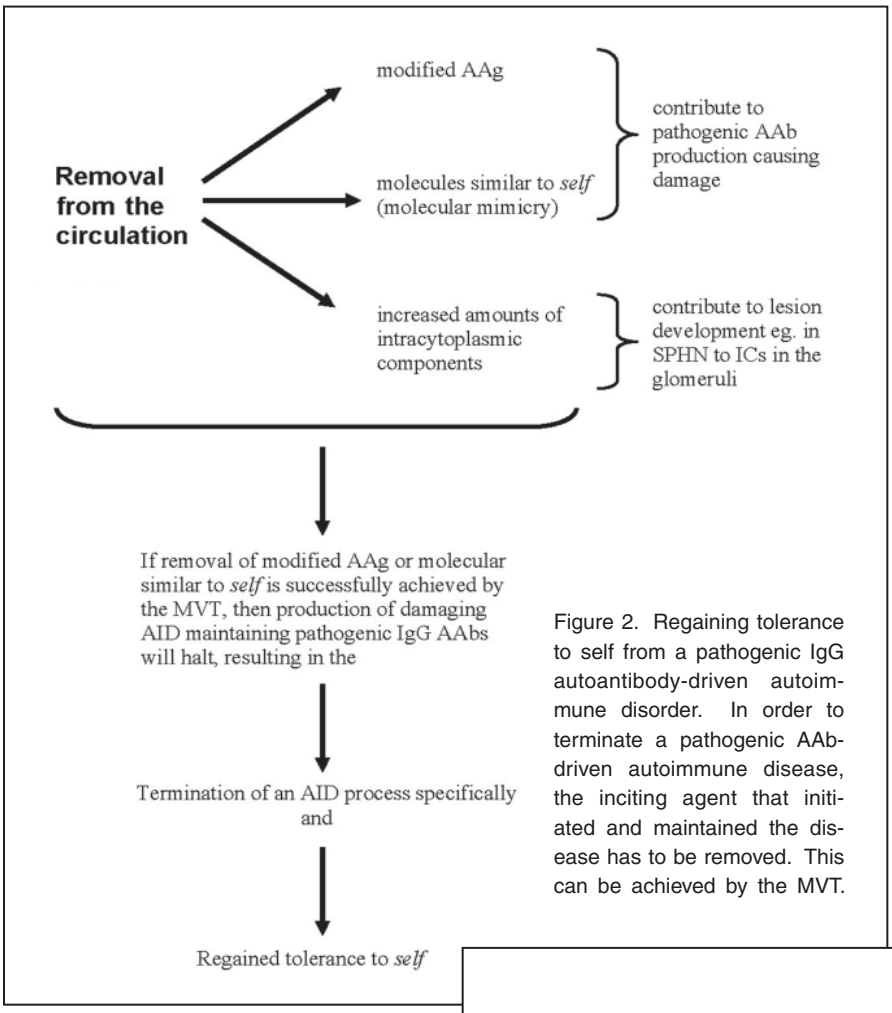

Figure 2. Regaining tolerance to self from a pathogenic IgG autoantibody-driven autoimmune disorder. In order to terminate a pathogenic AAb-driven autoimmune disease, the inciting agent that initiated and maintained the disease has to be removed. This can be achieved by the MVT.

third of the major vaccination techniques, after active and passive immunization. It is called modified vaccination technique (MVT, [patent in process]). MVT is able to correct mishaps caused by abnormal presentation of endogenous Ags both prophylactically and therapeutically with equal effectiveness, and it does so specifically and without causing side-effects. We describe in this communication why and how our vaccination technique holds the promise of preventing or terminating chronic ailments that so far have only been treatable by drugs.

Autoimmunity

Autoimmunity encompasses a complex network of immune responses primarily aiming to benefit the host by preventing the occurrence of autoimmune disorders and cancer.

The immune system faces two sources of Ags, one being the outside world (bacteria, viruses, etc.), and the other being our internal environment where there are, for example, AAgs released from damaged cells, as well as modified native Ags (*e.g.,* chemically modified self, molecules that are self-like through molecular mimicry, and cancer-specific Ags on cancer cells).

Throughout life—in fact from birth—released intracytoplasmic antigenic components from intact cells damaged by factors such as ischemia, trauma, burns, toxins (including drugs), and radiation are assisted in their removal by specific nonpathogenic IgM autoantibodies (AAbs).[13,24-27] Normal cells coming to the end of their life span are also included in this list. The final degradation of the intracytoplasmic Ags into reusable, small molecular weight (MW) Ags takes place in mononuclear cells, macrophages, mesangial cells, etc.[28,29]

Some of the degraded antigenic components also stimulate specific nonpathogenic IgM AAb cell lines to produce IgM AAbs to keep the level of circulating IgM AAbs at a constant

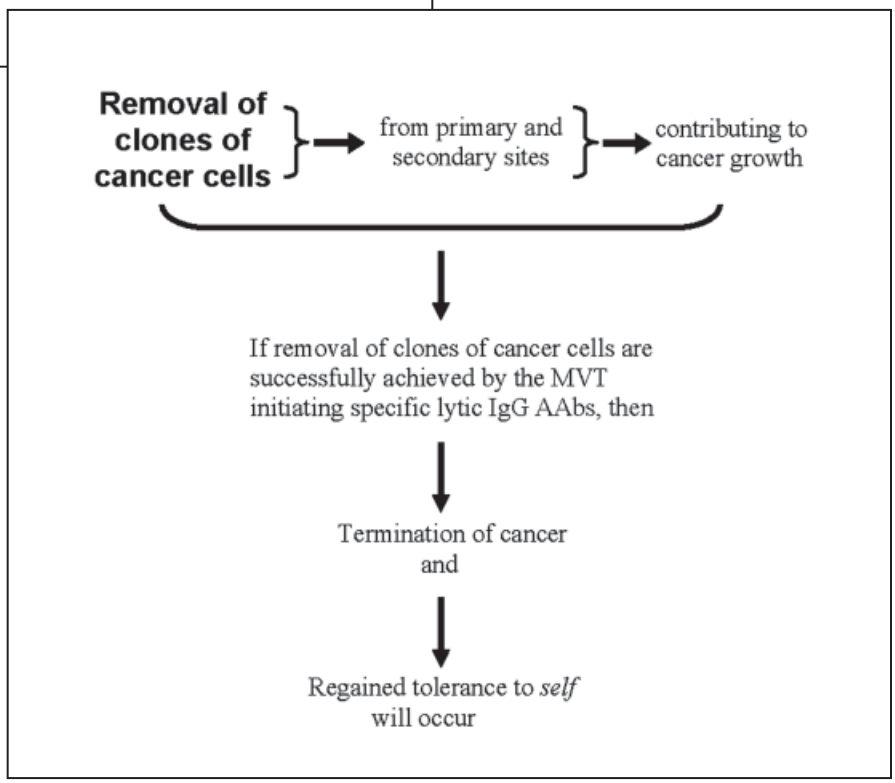

Figure 3. Regaining tolerance to self from cancer. In order for the body to regain normalcy from cancer, we have to initiate and maintain the production of specific lytic IgG AAbs against the cancer-specific Ags on cancer cells.

level.[27] Since throughout our lives our cells are continually damaged by outside influences, released AAgs are regularly present in our internal environments, ready for degradation, and also stimulating the ceaseless production of IgM AAbs. In a physiological sense, we are intolerant to subcellular components residing in the intracellular space of our own bodies.[30]

And in a pathogenic sense, we should not be tolerant to cancer-specific Ags that reside on the outer surfaces of emerging cancer cell clones. Therefore, a second beneficial effect of the autoimmune network should be directed against clones of cells that bear cancer-specific Ags. Once Ags residing on cancer cells are recognized as unwanted or modified self, the immune system should produce a pathogenic lytic IgG AAb response against the Ags and cause the cancer cells to lyse.

Pathogenic IgG AAb response, whether beneficial (cancer elimination) or harmful (autoimmune disease causing) will only occur if a self-AAg is presented to the cells of the immune system in a relatively modified form. For example, an autoimmune disease can start when an AAg is modified by a chemical agent or drug and appears to immune cells as a hapten protein conjugate.[31-34] Such modified self-AAgs will initiate, and if continuously present, maintain the production of pathogenic IgG AAbs. Therefore, in a progressive autoimmune disease, IgG Abs will remain in the circulation and react with the modified AAgs that caused their formation. They will also react with the normal target AAg—within a tissue or organ (cross-reactivity) where the native AAg resides—and will cause organ damage resulting in functional and morphological disturbances of the target organ (Figure 4).[35,36]

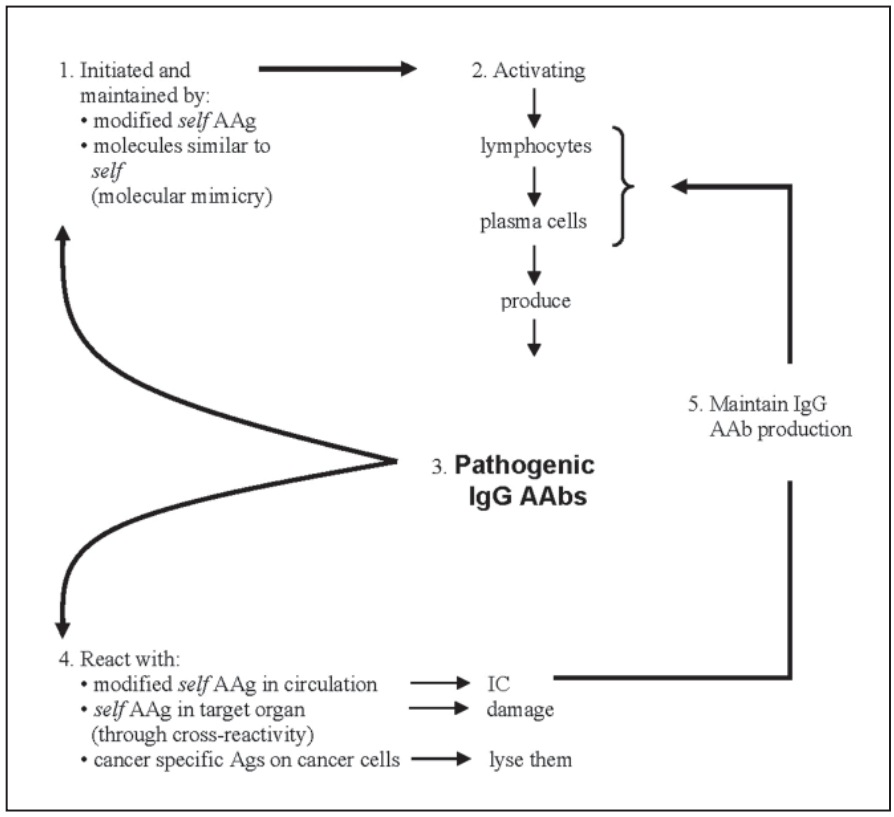

Figure 4. Initiating and maintaining a pathogenic autoantibody response. Pathogenic immune response is initiated and maintained by abnormal presentation of self (1, 5). Activated lymphocytes and plasma cells (2) produce pathogenic IgG AAbs (3) that can cross-react with target Ags (4) and cause damage.

Antigen-Specific Immunotherapy

The cells of the immune system are able to respond specifically to Ag presentation. In fact, the presentation of the Ag to the cells of the immune system determines the immune response outcome. We know—in dealing with a number of diseases involving viral and bacterial infection—how to present exogenous Ags in live, attenuated live, or dead forms, with or without adjuvants, etc., to evoke powerful protective immune responses. While numerous exogenous Ag-initiated diseases have been effectively controlled, endogenous Ag-provoked disorders (autoimmune disorders, cancer) could not—until recently—be specifically prevented or treated using existing vaccination techniques.

The exception, as far as dealing with endogenous Ag-derived disorders goes, is a vaccination technique using soluble tissue Ags delivered by oral, nasal, or intraperitoneal (IP) routes that is able to prevent the development of certain autoimmune diseases.[19,37-39] The same technique employed therapeutically, however, has not resulted in acceptable health benefit outcomes in most instances.[9,40-42] The reason why endogenous Ag-initiated disorders have not been treatable is because we have not fully understood the etiology and pathogenesis of most autoimmune disorders; and the same is true of cancer.[43-45] Even in cases where we know the etiological agents, we do not know how to present the AAgs to initiate and maintain appropriate immune responses. These AAgs must specifically downregulate pathogenic autoimmune-causing events in autoimmune disorders, or specifically upregulate immune responses against cancer Ags to kill cancer cells with pathogenic lytic IgG AAbs.

In order to initiate a corrective immune response in a host to prevent or treat endogenous Ag-induced autoimmune diseases and cancer, the following factors must be considered:

- the full understanding of "autoimmunity" as it relates to the particular disease;

- the availability of pure target Ag(s) against which the desired immune response is needed to downregulate

or upregulate pathogenic immune events;

• the availability of specific (nonpathogenic or pathogenic) Ab(s) against the target Ag(s);

• the vehicle through which Ag presentation is made—in this regard—the application of the MVT is paramount to achieving desired health benefit outcomes specifically, and without side-effects.

Regaining Tolerance to Self in Slowly Progressive Heymann Nephritis

We and others have extensively studied various aspects of Heymann nephritis, an experimental autoimmune kidney disease in rats.[46-52] For this disease, we have described the immunopathological processes that are responsible for disease development and maintenance.[28,31,35,53] We have also shown that during disease, two autoimmune events take place:

• A pathogenic immune response results in initial formation of immune complexes (ICs) in the glomeruli and tubules (primary immune response) by the developing pathogenic IgG AAbs directed against the target nephritogenic Ag. The continuously developing pathogenic IgG AAbs cause major structural alterations by IC depositions in the glomeruli (causing secondary IC depositions) and direct damage to brush border (BB)-related AAgs.

• A nonpathogenic immune response takes place consisting of increased levels of specific IgM AAbs (secondary immune response) whose aim is to remove from the circulation AAg (modified AAg) that maintain and contribute to the progression of SPHN (released self-AAg from the renal tubules).[14,31,54]

What fuels progressive lesion development in SPHN can be explained as follows:

• Modified self-Ags stimulate the development (primary immune response) and continuance (secondary immune response) of pathogenic IgG AAb formation.

• Pathogenic IgG AAbs react with glomerular padocyte fixed nephritogenic Ags that are trapped at these sites by specific IgM AAbs (resulting in the initial formation of ICs) and also react with the BB-related nephritogenic AAgs.[28]

• Pathogenic IgG AAbs reacting with the BB region, a rich zone of nephritogenic Ags, cause damage and the release of nephritogenic Ags into the urine and circulation.[14,54-61]

• Some of the released nephritogenic AAgs are assisted in their removal by specific IgM AAbs and mononuclear cells including macrophages, mesangial cells, etc.[14,31,54,56,62]

• Some of the nephritogenic AAgs stimulate further production of specific IgM AAbs (directed against the nephritogenic AAg—secondary immune response).[31]

• Some of the circulating nephritogenic AAgs will contribute (together with the circulating pathogenic IgG AAbs and C5b-9 membrane attack complex) to *in situ* IC depositions, growths, and enlargements on the epithelial side of the glomerular basement membrane (resulting in secondary IC depositions).[35]

As long as pathogenic IgG AAb production continues, the progression of the autoimmune disease will be maintained (Figure 4).[36,54] To terminate autoimmune disease-causing events that are responsible for continued pathogenic IgG AAb formation, both modified (pathogenic IgG AAb maintainer) and native (IC contributor) nephritogenic AAgs have to be excluded from the circulation (Figure 2). This can be achieved by our new vaccination technique, MVT.[12,14,54]

MVT is able to specifically remove from the circulation:

• the disease-maintaining modified nephritogenic Ag responsible for pathogenic AAb production through increased levels of specific IgM AAbs that cross-react with the modified nephritogenic Ag;

• the native nephritogenic Ag released from the BB region of the tubules that contributes to glomerular IC deposition (also by increased levels of specific IgM AAbs).

The lack of modified or native AAgs in the circulation allows tolerance to the native nephritogenic Ag to be regained, though immunological memory to the modified Ag is retained (Figure 2).

Components of the Modified Vaccination Technique

The modified vaccine preparation is made up of two components for the prevention and treatment of SPHN:

• native nephritogenic Ag; and

• specific homologous IgM Ab against the nephritogenic Ag.

The vaccine is made by mixing the two components together at slight Ag excess to obtain ICs. Injection of ICs in the host produces the same class of Ab with the same specificity against the target Ag as is present in the inoculum.[12,63] In the case of SPHN, elevated levels of rat anti-rat nephritogenic Ag IgM AAbs are present in the circulation. The function of these AAbs is physiologic. Through cross-reactivity, specific IgM AAbs assist in the removal of both native and modified self-AAgs from the circulation.[12,56]

No circulating modified nephritogenic AAgs in the circulation means no further stimulation of pathogenic IgG AAb cell lines to produce damaging pathogenic IgG AAbs. And no native AAgs in the circulation results in no further deposition of nephritogenic AAgs (together with the pathogenic IgG AAbs) in the glomeruli. Through such manipulated immune responses, tolerance to self (*i.e.*, to nephritogenic AAg) is re-established (though memory is retained to modified nephritogenic AAgs).

The MVT has many potential benefi-

cial properties, for example:

• re-establishing tolerance to self without the use of drugs, specifically and without side-effects, through enhancement of the immune system's normal functioning;

• prepared ICs evoking in animals or human patients a predetermined Ab response by Ab information transfer—recipients produce the same Ab with the same specificity against the target Ag as that which resides in the IC;

• evoking a secondary Ab response-like immune event (as if the immune system already had knowledge of producing the required immune response);[63] and

• achieving preventative and curative responses of AAb initiated and maintained autoimmune disorders.

The MVT also holds the promise, with appropriate modifications, of preventing and curing cancer and diseases caused by chronic infection as well.

Challenges for the Implementation of the Modified Vaccination Technique for Preventing and Treating Chronic Ailments

The following challenges remain for the implementation of the MVT:

• Etiological factors that cause autoimmune disorders through modified self or molecular mimicry have not yet been identified, in many instances.

• As well, immunological events that are responsible for the disease and also those processes that can downregulate autoimmune disease-causing events are, in many cases, not known.

• In the future, it will be essential to prepare *ex vivo*, by various chemical procedures, safe, pure, reliable, and efficacious AAg-equivalent components that are needed in the MVT to prevent and treat pathogenic AAb-initiated and maintained autoimmune disorders.

• For autoimmune disorders, it will be essential to produce specific IgM Abs against the various native AAgs that could be targets and also contributors of lesion development.

• For cancer and chronic infections, tumor-specific Ags as well as epitopes related to various chronic infectious disorders must also be produced. These Ags would be targeted by the MVT to induce preventative or therapeutic immune responses against the disease agents.

• Additionally, specific pathogenic Abs against the Ags must be prepared by monoclonal Ab techniques.

Specific Ags (AID-contributing, cancer-specific, etc.) and specific Abs against the target Ags are prepared, and the immunizing materials are mixed at slight Ag excess; only then can the modified vaccine be prepared.

Conclusion

Through the MVT, specific prophylactic and therapeutic applications could be realized in the very near future to correct endogenous Ag-initiated and maintained mishaps. The MVT could provide specific preventative and curative applications in both autoimmune disorders and cancer without the use of presently employed immunosuppressive and chemotherapeutic agents. The MVT requires *ex vivo* preparations of pure, safe, and efficacious Ags. They must be equivalent in their chemical structures and properties with endogenous Ags, and specifically-produced humanized monoclonal Abs (MAbs) against the designated Ags. Through the application of the MVT, tolerance to self could be re-established safely in the shortest possible time by our exploiting the immune system's ability to respond to the "information" contained in the vaccine. Through active immunization, the MVT evokes a predetermined immune response outcome. The injected host produces the same class of Ig, with the same specificity against the target Ag, as resides in the inoculum.[12,23,63] In our opinion, such a readjustment back to a normal state cannot be achieved by recently advocated passive immunization techniques. Passive immunization is costly and would serve as a therapeutic intervention in fewer patients because of the large volumes and frequent injections of specific Abs required.

Active immunization using the MVT appears to be the most powerful known immune response inducer against both exogenous and endogenous Ags without the use of adjuvants. We believe the MVT will eventually be used for preventative and therapeutic interventions in a wide range of acute and chronic infections. MVT will also be appropriate for endogenous Ag-caused disorders (especially in the very young and old, and in immune-compromised patients).

The prevention and termination of Ag-specific diseases that are currently only treatable with drugs is now in sight. We have shown most conclusively that an experimental autoimmune kidney disease (SPHN) can be prevented, and with equal effectiveness, terminated in 100% of rats through the appropriate application of the MVT. We call the new immunization method "MVT" since in every disease condition, the technique has to be modified specifically to achieve tailor-made Ab responses. We have mentioned that at the present time, we would be unable to vaccinate against all autoimmune disorders and cancers using the MVT because, in many instances, the etiological agents are not yet defined. We need to discover and procure the specific antigenic components that contribute to the initiation and maintenance of each disease. Hopefully, concerted efforts will be dedicated in the near future to achieving this attainable task.

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