Glatiramer Acetate: from Bench to Bed and Back

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ABSTRACT: Glatiramer acetate (GA, Copaxone®, Copolymer1, Cop 1) is an approved drug for the treatment of relapsing-remitting multiple sclerosis (RRMS). Its efficacy in reducing the frequency of exacerbations and its safety profile establish it as a first-line therapy for MS. Evidence from the animal model experimental autoimmune encephalomyelitis (EAE) and from MS patients indicates that GA affects various levels of the innate and the adaptive immune response, inducing deviation from the pro-inflammatory to the anti-inflammatory pathways. This includes mainly the induction of Th2/3 and T-regulatory cells, and down-regulation of both Th1 and Th17 cells. The immune cells induced by GA reach the CNS and secrete in situ anti-inflammatory cytokines, alleviating the pathological processes. In addition to its immunomodulatory activities, GA promotes neuroprotective repair processes such as secretion of neurotrophic factors, remyelination and neurogenesis, indicating that the repair process in the CNS can be up-regulated by therapy.

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Research in academic institutions is mostly basic research, namely curiosity-driven. In some cases, however, it reaches a stage when it becomes applicable. In these cases it is of utmost importance to follow it to its full potential. One example is the development of Copaxone, which started as a basic research project and culminated as a drug that benefits hundreds of thousands of MS patients.

HISTORY OF DEVELOPMENT

The development of glatiramer acetate (Copaxone®, Teva, Israel) began in the late 1960s as a basic research project intended to study the mechanisms involved in the induction of experimental autoimmune encephalitis (EAE), which is the primary animal model for MS. This is an induced neurological autoimmune disease mediated by autoreactive T-cells that recognize the encephalitogenic antigen(s) in association with major histocompatibility complex (MHC) class II molecules, which migrate into the central nervous system (CNS) and mediate the pathogenic process. Three main myelin proteins were demonstrated to be encephalitogenic: myelin basic protein (MBP), proteolipid protein (PLP), and myelin oligodendrocyte glycoprotein (MOG). These three proteins or their corresponding peptides were also implicated as putative autoantigens in MS [1]. However, when our research began in 1967, the only encephalitogenic material identified in the CNS was MBP.

Our approach was to use synthetic copolymers of amino acids whose composition resembled that of MBP, assuming that they would simulate the ability of MBP to induce EAE, thus serving as a research tool for studying the mechanism of induction of the disease. However, none of the synthesized copolymers proved to be encephalotogenic. On the contrary, some of them, particularly copolymer 1 (Cop-1), showed high efficacy in suppressing both incidence and severity of EAE [2]. These results led to a shift of direction in our research – towards the study of disease suppression, and eventually to the development of the drug.

In our pre-clinical experiments, in guinea pigs, the incidence of EAE was reduced from 75% in the control group to 20% in those treated with Cop 1, results that were reproducible with a different but identical batch of Cop 1. Further experiments demonstrated that Cop 1 is effective in suppressing EAE also in several other animal species, including rabbits, mice and primates [3], with a remarkable degree of suppression ranging between 60% and 100%. The studies in primates are of particular relevance to the treatment of MS in humans. It was known that rhesus monkeys and baboons were very sensitive to MBP-induced EAE and typically died within 2 weeks of the onset of symptoms. Treatment with Cop 1 was found to reverse EAE in both species when administered after the appearance of symptoms [4]. Furthermore, Cop 1 was effective not only in the acute model of EAE but also in the chronic relapsing model (CR-EAE), which is characterized by two or more discrete periods with clinical or neurological signs and resembles the appearance of clinical relapses in relapsing-remitting multiple sclerosis (RRMS). Cop 1 was effective in both preventing and suppressing CR-EAE induced in juvenile guinea pigs [5].

In view of the putative resemblance between EAE and MS and the assumption that MBP may be involved in the pathogenesis of MS, preliminary clinical trials using Cop 1 were conducted in MS patients. These were begun after toxicity studies in experimental animals showed that Cop 1 was non-toxic after both acute and sub-chronic administration to

mice, rats, rabbits and beagle dogs. Our clinical trials included three preliminary open trials and two double-blind phase II trials, one involving exacerbating-remitting (ER) patients and another with chronic progressive (CP) patients. These were followed by a Phase III trial.

The first preliminary trial was performed by Prof. Oded Abramsky at Hadassah Medical Center in Israel and included four patients in terminal stages of MS with severe disabilities. They received 2 mg Cop 1 daily for the duration of 5 months. All four were stable during treatment; Cop 1 was well tolerated and no side effects or toxicity were observed [6]. The second open trial was performed by Dr. Helmut Bauer in Gottingen, Germany, involving 21 patients, 10 of them receiving 2 mg Cop 1 daily and 11 receiving 20 mg daily, for 1 month. Its importance is the further demonstration of safety: only a few minor local reactions were observed [7]. The third preliminary trial, performed by Dr. Murray Bornstein at the Einstein College of Medicine in New York, included 16 MS patients, 12 with chronic progressive disease and 4 with RRMS. Each received 20 mg Cop 1 for the duration of 6 months. At the end of the study, 2 of the 4 RRMS patients and 3 of the 12 CP patients showed improvement, showing reduced numbers of relapses or slowed progression [8].

The first phase II trial was double blind, performed at the Albert Einstein College of Medicine, and included 48 RRMS patients, pair-matched, 24 in each arm, and lasted for 24 months. The average number of relapses per patient in 2 years dropped from 2.7 in the control group to 0.6 in those treated with Cop 1. This was accompanied by a difference in the EDSS score (0.75 units) [9]. A second phase II double-blind trial, with chronic progressive patients, was performed in two centers – the Albert Einstein College of Medicine in New York and the Baylor College in Houston. It included 169 patients and lasted for 24 months. The results showed a trend for less progression in the groups receiving Cop 1 compared to the placebo (17.6% versus 25.5%) [10].

Based on these cumulative results, Teva Pharmaceuticals Ltd (Israel) undertook the development of Cop 1 as a drug, and performed a Phase III trial, which was a double-blind placebo-controlled multicenter trial involving 251 exacerbating-remitting MS patients in 11 medical centers in the United States. The enrolled patients had clinically definite MS (EDSS 0-5) with two or more well-defined relapses in the 2 years prior to randomization. They were randomly assigned to receive daily subcutaneous injections of 20 mg Cop 1 or placebo control. At the end of 24 months an overall reduction of 29% in relapse rate was observed in the Cop 1 group compared to the placebo (P = 0.007), with some beneficial effect in the disability status in favor of Cop 1 (P = 0.024). Furthermore, the positive effects of Cop 1 on neurological disability persisted during a 9-month extension of this trial. Most importantly, the side effects observed were minimal, consisting mainly of mild injection site reactions [11]. A subset of the patients participated in a pilot MRI study, and the percent of patients who had static or improved scan versus worse scans also favored Cop 1 treatment.

Based on the successful results of the Phase III trial and the cumulative results of the two double-blind Phase II trials, Copolymer I was submitted to the FDA under the commercial name Copaxone®. Approval for the treatment of RRMS was obtained in December 1996 and the drug has been marketed worldwide since 1997. It is also known by the generic name, glatiramer acetate (GA).

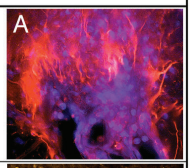
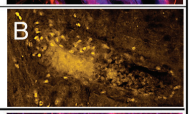
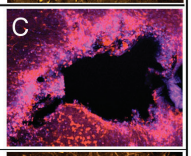
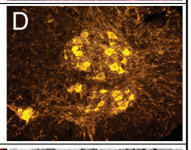
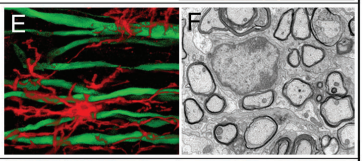
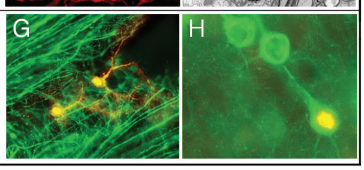
A large group of patients, who participated in the Phase II and Phase III trials and continued to receive GA, were followed for a period of 15 to 22 years [12,13] during which they were evaluated every 6 months. The results show that the annual relapse rates declined from 1.18 pre-study to approximately 0.2 after 10 years, and remained steady, or improved, reaching 0.12 after 15 years. Mean EDSS change was 0.50 points and 57% of the patients had stable/improved EDSS scores. Furthermore, patients who withdrew from the study had greater disability than the ongoing patients. These data provide clear evidence for the long-term efficacy and high safety profile of Copaxone. In parallel to the pharmacological development, extensive efforts in our laboratory were devoted to elucidating the mechanism of activity of Copaxone, as detailed below.

GA, Copaxone®, a synthetic random copolymer of amino acids, is an approved drug for the treatment of MS since 1997, administered by daily injection of 20 mg or 40 mg three times weekly

IMMUNOMODULATORY MECHANISMS

GA induces a broad immunomodulatory effect on various subsets of the immune system [Figure 1]. The initial prerequisite step is the binding of GA to major histocompatibility (MHC) class II molecules. In vitro studies on murine and human antigen-presenting cells (APCs) showed that GA undergoes a rapid and efficient binding to various MHC class II molecules and even displaces other peptides from the MHC binding groove [14]. This competitive binding for the histocompatibility molecules can prevent the presentation of other antigens and hinder their T-cell activation. Several groups have demonstrated that GA induces generalized alterations of various types of APCs, such as dendritic cells and monocytes, so that they preferentially stimulate protective anti-inflammatory responses [15]. This modulation at the level of the innate immune system is the least specific step in the immunological processes affected by GA and can be beneficial for inhibiting the response to several myelin antigens. In addition, GA acts in a strictly antigen-specific manner in the case of the MBP immunodominant encephalitogenic epitope (comprising amino acids 82-100). Using MBP 82-100 specific T-cell clones from MS patients and from EAE-induced mice, it was shown that GA inhibits their

Figure 1. Immunomodulatory and neuroprotective effects of glatiramer acetate in MS and EAE. Inserts demonstrate the in situ consequences of GA in the CNS:
[A] GA-specific T-cells (blue) expressing IL-10 (red)
[B] Infiltration of Foxp3 expressing T-cells (yellow)
[C] GA-specific T-cells (blue) expressing BDNF (red)
[D] Preservation of motor neurons
[E] Oligodendrocyte progenitor cells (red) extending processes between transected neuronal fibers (green)
[F] Remyelination zone with newly myelinated axons surrounding an oligodendrocyte
[G] Neuronal progenitor cells (yellow) in a lesion site following GA treatment
[H] A BrdU expressing neuron (yellow) born during BrdU/GA injection, also expressing a mature neuronal marker in the cortex (green).

Peripheral immunomodulation	Competition for MHC	Promiscuous binding to various MHC class II molecules, displacement of myelin antigens from the MHC binding groove.	
	Alteration of the innate immune response	Inhibitory effect on monocytes reactivity, deviation of dendritic cells and monocytes to produce less TNF-α and IL-12, more IL-10 and TGF-β, and to stimulate Th2 anti-inflammatory responses.	
	T-cell receptor antagonism	Inhibition of the activation of T-cells specific to the 82-100 epitope of MBP.	
	T-cell deviation	Induction of specific Th2/3-cells that secrete high amounts of IL-4, IL-5, IL-10, and TGF-β. Elevation of the prevalence and function of T-regulatory cells, activation of the transcription factor Foxp3. Reduction of Th-17 cells and their transcription factors RORγt. Improvement of the regulatory function of $CD8^+$ T-cells.	
	Modification of B-cells	Induction of antibodies with beneficial rather than neutralizing activity. Bias towards production of anti-inflammatory cytokines such as IL-10. Down-regulating of chemokine receptors.	
Immunomodulation in the CNS	Secretion of anti-inflammatory cytokine	GA-specific Th2/3 cells cross the BBB and secrete *in situ* anti-inflammatory cytokines. Bystander expression of IL-10 and TGF-β by resident astrocyte and microglia. Reduction in the overall expression of IFN-γ.	
	Th-17 and T-regulatory cells	Decrease in the amount of Th-17 cells. Increase in T-regulatory cells.	
Neuroprotection	Elevation of neurotrophic factors	GA-specific T-cells express BDNF in the brain. Restoration of the impaired expressions of BDNF, NT-3, NT4, IGF-1, and IGF-2.	
	Reduced CNS injury	Prevention of demyelination. Preservation of retinal ganglion cells. Inhibition of motor neuron loss. Preservation of brain tissue integrity by the MRI parameters MTR and DTI. Reduced formation of "black holes" Increase in NAA:Cr ratio.	
	Remyelination	Augmented remyelination. Increased proliferation, maturation and survival of oligodendrocyte progenitor cells and their accumulation in the lesions.	
	Neurogenesis	Elevated proliferation, migration and differentiation of neuronal progenitor cells and their recruitment into injury sites.	

activation by T-cell receptor antagonism, acting as an altered peptide ligand [16].

Most studies attribute the primary mechanism of GA activity to its ability to shift the T-cell response from the pro-inflammatory to the anti-inflammatory pathway. It has long been known that GA-treated animals develop specific T-cells in their peripheral immune system [17]. These cells act as modulatory suppressor cells, as they inhibit the response of MBP-specific effector cells in vitro and adoptively transfer protection against EAE in vivo. T-cell lines induced by GA progressively polarize towards the T-helper (Th) 2/3 subtype, secreting high amounts of anti-inflammatory cytokines such as interleukin (IL) -4, -5, -10, and transforming growth factor-beta (TGFβ) [17]. A shift from a pro-inflammatory Th1-biased cytokine profile towards an anti-inflammatory Th2-biased profile was also observed in GA-treated MS patients [18,19], indicating that such cells are involved in the therapeutic effect of GA in MS.

Several studies, in EAE-induced mice, demonstrated the effect of GA on Th17 and on Treg cells, which are pivotal effectors of disease exacerbation and suppression, respectively [20,21]. In vitro exposure of peripheral $CD4^+$ T-cells, from healthy humans or from GA-immunized mice, to GA results in elevated level of Tregs, through activation of the transcription factor forkhead box P3 (Foxp3) [22]. Furthermore, GA treatment leads to increased Foxp3 expression in $CD4^+$ T-cells of MS patients, whose Foxp3 level is low at baseline. In addition to its effect on the $CD4^+$ T-cell subset, GA affects $CD8^+$ T-cells. The regulatory function of these cells, which is impaired in MS untreated patients, is drastically improved after several months of GA treatment to levels observed in healthy individuals [23]. The effect of GA on B-cells also contributes to its therapeutic activity, by biasing towards anti-inflammatory cytokines such as IL-10 [24].

The efficacy of GA in reducing the frequency of exacerbations and its high safety profile establish this drug as a first-line treatment for multiple sclerosis

A therapy's efficacy is obviously measured by its consequence in the disease organ, in the case of an MS therapy by its ability to modulate the pathological processes in the CNS. Specific ex vivo reactivity to GA, manifested by cell proliferation and by Th2 cytokine secretion, was found in whole lymphocyte populations isolated from brains of EAE-induced mice treated with GA [25]. Moreover, highly reactive GA-specific T-cell lines, which secrete IL-4, IL-5, IL-10 and TGFb in response to GA, and cross-react with MBP at the level of Th2 cytokine secretion, were obtained from brains and spinal cords of GA-treated mice. The ability of the GA-induced cells to cross the blood-brain barrier and accumulate in the CNS was confirmed by the injection of labeled GA-specific T-cells into the periphery and their subsequent detection in the brain [25]. In the CNS of EAE-induced mice, GA-specific T-cells highly express the potent regulatory anti-inflammatory cytokines IL-10 and TGFb [26]. Of special interest is the finding that IL-10 and TGFb are expressed not only by the GA-specific T-cells but also by CNS resident cells in their vicinity, such as astrocytes and microglia. In addition, in mice with either chronic or relapsing-remitting EAE, GA treatment results in a drastic reduction in the pro-inflammatory Th17 cells, and a parallel elevation of Tregs in the CNS [21]. Importantly, analysis of GA-reactive T-cells from the cerebrospinal fluid (CSF) of GA-treated MS patients revealed a pronounced anti-inflammatory profile [27]. These cumulative results indicate that GA induces a bystander immunomodulatory effect in the CNS and generates an in situ anti-inflammatory cytokine shift, thus restraining the pro-inflammatory pathological disease progression.

NEUROPROTECTION AND REPAIR PROCESSES

An essential challenge for MS therapy is to target not only the inflammatory characteristic of the disease but also its neuroaxonal pathology, inducing neuroprotective outcomes. Accumulated findings indicate that GA treatment generates neuroprotective consequences in the CNS [Figure 1]. The first indication for neuroprotective activity was the ability of GA-induced cells to secrete brain-derived neurotrophic factor (BDNF). This was demonstrated for murine GA-specific T-cells originating from the periphery or the CNS, as well as for human T-cell lines [26,28]. Furthermore, GA-specific T-cells demonstrated extensive BDNF expression in the brain of EAE-induced mice [26]. BDNF was shown to be elevated in brains of mice that were injected daily with GA [29]. A similar elevation was found in brains of GA-treated mice for additional neurotrophic factors such as the neurotrophins NT-3 and NT-4 [29], as well as for insulin-like growth factor (IGF)-1 [30] and IGF-2 [31]·. Of special significance is the elevation by GA of levels of neurotrophic factors even when treatment starts late, in the chronic disease phase when their levels are diminished. Furthermore, in Mecp2 knockout mice in which BDNF levels are low, GA treatment induced elevation of BDNF expression to the level found in brains of healthy mice [32]. The relevance of this effect to human therapy has been shown in the reversal of the reduced BDNF levels in the serum and in the cerebral spinal fluid of MS patients, following GA treatment [33].

The neuroprotective effect of GA is manifested by actual preservation of the CNS and reduced typical tissue damage. Several studies, utilizing immunohistochemistry and electron microscopy in different EAE models, demonstrated the protective outcome of GA on the primary disease target, the myelin [4,34]. Furthermore, in mice inflicted with MOG-induced EAE, in which chronic disease with extensive neurodegeneration is typically manifested, GA treatment results in reduced neuroaxonal damage. This was evident by the preservation of retinal ganglion cells [35], less axonal deterioration, and fewer

deformed neurons [36]. Motor neuron loss that occurs in this model was also prevented by GA treatment [37]. In a study that employed MRI parameters such as magnetization transfer ratio (MTR) and diffusion tensor imaging DTI for the assessment of the whole brain as well as for the detection of specific affected regions, GA restored all the MRI parameters in both chronic and relapsing-remitting EAE models [38]. When treatment was applied before the appearance of clinical manifestations, blocking the development of the pathological processes (prevention regimen), mice displayed nearly no damage. Moreover, when treatment was applied in a therapeutic schedule, after disease exacerbation, when substantial injury was already manifested (suppression regimen), a significant reduction in myelin and neuroaxonal damages was obtained. This suggested the induction of genuine repair mechanisms.

The central elements of the CNS, the myelinating oligodendrocytes as well as the neurons, are terminally differentiated cells with a limited capacity to respond to injury. They depend for renewal on the availability of their precursors, the oligodendrocyte progenitor cells (OPCs) and the neuronal progenitor cells (NPCs), which need to undergo proliferation, migration and differentiation into the defined progeny. Repair processes are characteristic mostly of the early MS and EAE phases. As the disease progresses, progenitor cells succumb to the hostile conditions within the inflamed lesions, and self-repair mechanisms drastically decline. Promoting repair beyond the body's limited spontaneous extent is therefore a major goal of MS therapy.

The unique mechanism of action of GA involves immunomodulation and neuroprotection, as well as repair processes including remyelination and neurogenesis

Applying transmission electron microscopy (TEM), which facilitates the visualization of newly myelinated axons, we demonstrated the ability of GA to augment remyelination [37]. Ultrastructural quantitative analysis in the spinal cord of mice induced with relapsing-remitting EAE provided evidence for a significant increase in remyelination after GA treatment. The mode of action of GA in this system is attributed to an increase in proliferation, and survival of OPCs and their recruitment into injury sites [4,31]. In a recent study we investigated whether GA can affect postnatal myelinogenesis in the developing nervous system, when injected at postnatal days 7–21 [39]. Immunohistological and ultrastructural analyses revealed significant elevation in the number of myelinated axons as well as in the thickness of the myelin encircling them, in spinal cords of GA-injected mice compared to their PBS-injected littermates. A prominent elevation in the amount of OPCs and their proliferation, as well as in mature oligodendrocytes, indicated that similar to the findings in EAE the effect of GA in postnatal myelination is linked to increased proliferation and differentiation along the oligodendroglial maturation cascade. Furthermore, GA-injected mice exhibited better performance in a rotating rod test than their PBS-injected littermates, suggesting that the accelerated myelin development results in functional advantage in sensorimotor functions [39].

Concerning the functional cells in the CNS, the neurons, GA treatment augmented NPC proliferation to a higher level than that observed in EAE mice, and this effect persists for a prolonged duration [36]. Indeed, neuronal progenitors were seen diverging from the classic migratory streams and spreading to damage sites in adjacent brain regions that do not normally undergo neurogenesis. Findings from human studies support the notion that GA confers neuroprotection in MS patients. GA treatment reduced the formation of permanent T1 hypointense lesions that evolve into "black holes," which have been associated with irreversible neurological disability [40]. Using quantitative MRI analysis, it was also shown that GA treatment for 1 year leads to a significant increase in the NAA:Cr ratio compared to pre-treatment values, implying axonal metabolic recovery and protection from sub-lethal axonal injury [41]. Altogether, these findings may explain the long-term beneficial effect of GA in patients followed for 15 years and more [12,13].

The ability of GA to induce a neuroprotective growth-promoting environment supported its application for improving stem cell engraftment in the CNS. Towards this aim we inoculated (intraventricularly or intrapeitoneally) non-neural progenitor cells from a myogenic origin into EAE-inflicted mice [42]. GA treatment enhanced the engraftment of muscle progenitor cells, and their number was threefold higher than in untreated mice. Furthermore, GA promoted the differentiation of the myogenic progenitor cells towards the neuronal pathway, resulting in increased numbers of donor cells expressing neuronal markers in the cortex and the hippocampus.

Several laboratories have reported the beneficial effects of GA in animal models of neuronal trauma, such as loss of retinal ganglion cells and crush injury of the optic nerve [43], as well as in models of the neurodegenerative diseases Parkinson's [44] and amyotrophic lateral sclerosis (ALS) [45]. These cumulative results indicate that GA may have neuroprotective and neurogenerative effects in a broader spectrum of neurodegenerative disorders.

POTENTIAL FOR ADDITIONAL IMMUNE-MEDIATED APPLICATIONS

In view of the similarity in the pathological mechanisms that mediate autoimmune diseases and the immunomodulatory mode of action of GA, its application for additional immune mediated pathologies has been studied. The first assessment of GA in ameliorating immune mediated pathology other than MS/EAE was performed in transplantation models. Using a murine model of lethal graft-versus-host disease (GVHD), we demonstrated that post-transplantation GA treatment significantly reduced the incidence, onset, and severity of disease,

resulting in improved long-term survival [46,47]. GA treatment postponed skin graft rejection in mice in various strain combinations across minor and major MHC barriers, and improved the function of grafted thyroids [47]. Furthermore, GA drastically reduced the cytotoxic activity toward host targets in the bone marrow transplantation model and the responses against the graft in the organ transplantation models. In both systems, GA inhibited the secretion of Th1 inflammatory cytokines and induced the secretion of Th2/3 anti-inflammatory cytokines, in accord with its mechanism of action in EAE/MS. Combined treatments of GA with various suboptimal doses of the immunosuppressive drugs, cyclosporin A (CyA) or FK506 (tacrolimus), significantly improved graft survival and function compared to the effect of each drug alone [48].

GA treatment ameliorated the pathological manifestations in several inflammatory bowel disease (IBD) animal models, including trinitrobenzene sulfonic acid (TNBS)-induced colitis, dextran sulfate sodium (DSS)-induced colitis, as well as in a spontaneous model in transgenic mice [49]. The beneficial effect of GA was demonstrated by a significant reduction in several characteristics of this disease, i.e., weight loss, intestinal bleeding, diarrhea, and colon damage, resulting in improved long-term survival of the treated mice. In all these models the detrimental pro-inflammatory response manifested by TNFα and IFNγ expression was decreased following GA treatment, whereas the anti-inflammatory TGFβ and IL-10 response was elevated. These effects could be achieved not only by GA treatment but also by adoptive transfer of GA-specific T-cells originating in GA-immunized mice [50]. Similar to their migratory pattern in EAE, upon transfer to DSS-induced mice, the GA-specific cells localized in the diseased organ, in this case inner layers of the colon, and secreted in situ TGFb, thus inducing immunomodulation at the site in which the pathological process occurs.

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Capsule

Trends in human papillomavirus vaccine Types 16 and 18 in cervical precancers, 2008–2014

The impact of human papillomavirus (HPV) vaccination has been observed in the United States through declining cervical precancer incidence in young women. To further evaluate vaccine impact, **McCLung** et al. described trends in HPV vaccine types 16/18 in cervical precancers, 2008–2014. The authors analyzed data from a five-site, population-based surveillance system. Archived specimens from women aged 18–39 years diagnosed with cervical intraepithelial neoplasia grades 2–3 or adenocarcinoma in situ (CIN2+) were tested for 37 HPV types. They described the proportion and estimated number of cases of CIN2+ by HPV-type groups over time. Trends in HPV16/18-positive CIN2+ were examined, overall and by vaccination status, age, histologic grade, and race/ethnicity, using Cochrane-Armitage tests. In 10,206 cases, the proportion and estimated number of cases of HPV16/18-positive CIN2+ declined from 52.7% (1235 cases) in 2008 to 44.1% (819 cases) in 2014 ($P < 0.001$). Declining trends in the proportion of HPV16/18-positive CIN2+ were observed among vaccinated (55.2–33.3%, $P < 0.001$) and unvaccinated (51.0–47.3%, $P = 0.03$) women; ages 18–20 years (48.7–18.8%, $P = 0.02$), 21–24 (53.8–44.0%, $P < 0.001$), 25–29 (56.9–42.4%, $P < 0.001$), and 30–34 (49.8–45.8%, $P = 0.04$); CIN2 (40.8–29.9%, $P < 0.001$) and CIN2/3 (61.8–46.2%, $P < 0.001$); non-Hispanic white (59.5–47.9%, $P < 0.001$) and non-Hispanic black (40.7–26.5%, $P < 0.001$). The authors conclude that from 2008 to 2014, the proportion of HPV16/18-positive CIN2+ declined, with the greatest declines in vaccinated women; declines in unvaccinated women suggest herd protection. The declining proportion of HPV16/18-positive CIN2+ provides additional evidence of vaccine impact in the United States.

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