Complement levels before and after dives with a high risk of DCS.

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Nyquist P, Ball R, Sheridan MJ. Complement levels before and after dives with a high risk of DCS. Undersea Hyperb Med 2007; 34(3):191-197. Background: Previously, complement activation has been associated with decompression sickness (DCS). However data, both in humans and in animals, are controversial. Hypothesis: Complement activation and depletion occurs after exposure to the hyperbaric environment and is associated with increasing risk of DCS. Methods: We obtained serological samples from 102 dives (120-300 feet of seawater) with a constant partial pressure of O₂ set at 1.3 ATA in thirty-five U.S. Navy diver volunteers. Blood was obtained within one hour of diving and within one hour of surfacing. Plasma was extracted and analyzed for complement depletion. The risk of DCS was estimated using a validated model of DCS risk. Results: Pre-post dive concentrations of C3a were significantly related to estimated risk of DCS (Figure 1), but the variation in predicted DCS explained by C3a was small (correlation co-efficient (r² = 0.19, p<0.0001). Conclusions: There was a reduction in total Ca3 levels in divers after exposure to dives with a high estimated risk of DCS. This decomplementation appeared to increase as the estimated risk of DCS increased.

INTRODUCTION

Complement acts on tissues by stimulating lysis of foreign cells, initiating inflammation, and attracting macrophages to the site of injury. Complement is activated by bubbles through an alternative pathway. The clinical effects of complement have been hypothesized to cause the symptoms of DCS. Any genetic variation in complement activity may explain variation in the clinical presentation of DCS between individuals. Ward, et al. hypothesized that individual susceptibility to complement activation could explain individual variability in susceptibility to decompressions sickness (DCS) (1, 2). This idea was tested by examining complement in the air-plasma interface prior to diving in animals and as well as diver volunteers and then measuring the bubble loads and using them to determine risk of DCS. They found that human divers with an increased C3a and C5a activation in the predive bubble tests were more susceptible to DCS (2). In 1993 Stevens, et al. reported an increase in serum complement levels in four divers with Type I DCS, increasing interest in the role of complement in DCS (3).

The complement hypothesis of DCS has been further tested in rabbits and humans with mixed results (1, 4, and 5). Broome, et al. utilized a complement inhibitor in a rat model of DCS to test the hypothesis that complement inhibition would also suppress the development of DCS. However, no reduction in DCS was observed in his animal model (6). Anti-C5a monoclonal antibody was unable to attenuate
the clinical effect of air emboli identified through observation of the nitric oxide mediated relaxation response in rabbits. Clinical changes in endothelial function were observed and not prevented by the anti-C5a antibody (7). Most of these studies used measurements of complement that were relatively unstable point estimates of complement activation.

Complement activation, as represented by des-arg-C5a, is highly variable and single point measurements are not accurate indicators of complement activation (8). A human trial with a stable nephelometer-based assay of red blood cell C3d binding was performed and no complement activation or depletion was detected (9). Other strategies have attempted to overcome the inherent instability of C3a and C5a by using IC3, a conformationally changed component of the C3 system. IC3 was observed to be reduced in human subjects after hyperbaric exposure, even though the total complement level was unchanged (10).

The role of complement in DCS remains unclear. While some have reported its depletion in the high pressure environment, no one has demonstrated a conclusive relationship between complement activation and clinical DCS or risk of DCS. This is due to the paucity of observed DCS, and the lack of human dive trials large enough to yield sufficient statistical power to conclusively demonstrate an effect of DCS risk on complement formation.

The “Human Decompression Trial with 1.3 ATA Oxygen in Helium” protocol was designed to develop a 1.3 ATA constant partial pressure of oxygen dive tables to be used by the Navy’s Explosive Ordnance Disposal divers for their closed circuit underwater breathing apparatus. The “Human Decompression Trial with 1.3 ATA Oxygen in Helium” protocol also provided an opportunity to evaluate complement activation in human diver volunteers after dives for which the risk of DCS could be modeled by a probabilistic model (11-15). The primary goal of our sub-study was to detect complement activation in a large number of dives with a high risk of DCS. The secondary goal was to obtain serum samples in the setting of clinical DCS. Unfortunately, no cases of DCS where captured in our portion of the study. Activated complement derivatives such as C3a, C4a, and C5a have very short half lives. Any reduction or increase in these components after a dive would be indicative of complement activation and transformation into these derivatives. So while no observed DCS cases occurred in our study population, we hoped to detect such changes by measuring complement prior to and after dives.

METHODS

This study was titled “Humorally Mediated Markers of Decompression” and was approved by the Naval Medical Research Institute committee for the protection of human subjects. It was a sub-protocol of “The Human Decompression Trial with 1.3 ATA Oxygen in Helium”. In the parent dive trial 78 U.S. Navy divers each participated in 1-23 dives (median 5 dives per diver) with at least 60 h between each dive (12). The “1.3 ATA” trial dives ranged from 120-300 feet of seawater (fsw) and the profiles selected for the “Humorally Mediated” sub-protocol are indicated in Table 1 (120-260 fsw). DCS was determined by clinical examination with three diving medicine physicians consulted to verify the diagnosis. DCS symptoms were to be consistent with those described in the USN Diving Manual (14) involving the limbs or central nervous system (CNS). All dives were conducted with a constant partial pressure of O₂ set at 1.3 ATA. The dilutant gas was helium. There were 48 cases of DCS in this trial: 26 cases of Type II (neurological) DCS and 22 “marginal” cases. These marginal cases where typified by non-descript physical symptoms
occurring after dives that did not meet the USN criterion for Type I or Type II DCS but were deemed by a panel of DMO’s to be medically relevant. None of these cases were captured in the “Humorally Mediated Markers of Decompression Study” data set. However, five of our volunteers experienced DCS from other dives in this series but not from dives in the marker study.

Thirty-five of the 78 divers that volunteered to participate in the 1.3 ATA study also enrolled in the “Humorally Mediated Markers” protocol. Divers included in the complement portion of the study completed between 1 and 5 dives with serological testing. Blood was collected before and after every dive while the diver was enrolled in the study. Many did not join the study until later in the trial, and many dropped out secondary to the discomfort or inconvenience of the blood sampling procedure. Blood was obtained within one hour of diving and within one hour of surfacing. The blood was placed in EDTA (Sardsett Monovette tubes) and serum was extracted using a standard centrifugation methodology (3300 rpm for 5 min.). The serum specimen was placed on ice and immediately placed in a –70°C freezer until analysis for a variety of components. C3a and C5a were determined using ELISA kits in a standard 64-well reader per the manufacturer’s protocol (Amersham Corp., Arlington Heights, IL) with good standard plots. C-reactive protein was measured per protocol at the National Naval Medical Center, Bethesda, MD.

If complement activation is related to DCS, one might posit a continuum of “decompression stress” dependent on the dive profile in which more complement is activated based on the decompression severity. Decompression stress lacks a precise measurable definition, so we used the calculated probability of DCS for a given profile as a surrogate for decompression stress. The Linear Exponential Multi-gas (LEM) probabilistic model of DCS risk was developed by estimating parameters of a hazard function relating dive profile to DCS outcome, using the method of maximum likelihood (16). Model parameters were estimated from a wide variety of He-O2 and N2-O2 dives and tested in the trial from which the study samples were taken (11). The risk estimates from the model were used as a stratifying variable in the analysis of complement levels.

The independent variable for the study

<table>
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<th>Depth (fsw)</th>
<th>Bottom Time (min.)</th>
<th>Total Decompression Time (min.)</th>
<th># of dives included (complement)</th>
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was risk of DCS. The dependent variables were the changes in pre- and post-concentration of C3a, C4a, C5a, and CRP. The change in these variables was calculated by subtracting the pre dive values from the post dive values. Regression techniques were employed to assess the relationship between DCS and changing pre- and post-concentrations of C3a, C4a, C5a, and CRP. All calculations were performed using SAS software (v9.1, SAS Institute, Cary, NC).

RESULTS

We obtained complete results for all parameters of 102 dives (35 divers) of the 472 total dives completed in the trial. In total, 48/472 (10%) cases of DCS were observed in the larger trial. No DCS cases were captured in the complement trial. The number of dives that individual divers contributed to the humoral markers study varied between 1 and 5 with an average of 2 per diver. The risk of DCS was calculated and compared to the change in concentration of C3a, C4a, C5a, and CRP. Pre-post dive concentrations of C3a were significantly related to the probability of DCS (Figure 1), but the variation in DCS explained by C3a was small as indicated by a small correlation coefficient in the linear regression analysis ($r^2 = 0.19$, p<0.0001). The relationship was an inverse one: the risk of DCS increased there was a decrease in the amount of post-dive C3a. No significant association between pre-post change in levels of C4a ($r^2 < 0.01$, P=0.28), C5a ($r^2 < 0.01$, P=0.18) and CRP ($r^2 < 0.01$, P=0.38) and increased risk of DCS was observed (Figures 2, 3, and 4).

Other calculations designed to portray the change in complement levels were completed as a post hoc analysis of the data set. These included the estimation of change in complement level as a percent of the pre-dive complement levels using a linear regression
model with the probability of DCS as the independent variable. Alternative independent variables included the change in DCS as a percent of the pre-dive complement levels and the post-dive complement levels. No relationship was detected in any of these models.

**DISCUSSION**

DCS is caused by the formation of bubbles resulting from a sudden and sharp reduction in ambient atmospheric pressure in body tissues supersaturated with inert gases (16). Bubble formation is thought to occur predominantly in the vasculature, although most symptoms involve the joints and CNS (17-20). The symptoms of DCS are varied (19, 20). In the early days of DCS research, symptoms were assumed to be the direct result of mechanical effects of bubble formation in the bloodstream and affected tissues. The distribution of classic DCS symptom types was related to the unique physiologic characteristics of the anatomy affected (17-21).

Increasing clinical knowledge of DCS has raised new questions about this disease. If the clinical symptoms of DCS were related to bubble formation, then an increased disease burden in patients with large bubble burdens would be expected. While Doppler studies demonstrate a statistically significant association between bubble load and DCS, often divers with large bubble loads do not develop DCS (22, 23). Clinicians often report that individuals continuously exposed to the high pressure environment appear to develop habituation, exhibiting disease less frequently as their exposure continues over time (24). Many clinicians have made the observation that certain individuals seemed to be resistant to DCS regardless of clinical exposure. Such resistance raises theoretical questions about the fundamental pathophysiology of DCS. A reasonable hypothesis is that biological mechanisms are involved in the pathophysiology of DCS that are activated by bubble formation, yet act independently of it. Complement activation could play a role in the pathophysiology of DCS; it is readily activated by bubbles and its sites of activation are in blood and tissues. Complement may induce symptoms of DCS by initiating tissue changes through immune-mediated mechanisms including cell lysis, inflammation, and leukocyte chemoattraction.

This study endeavored to establish a relationship between increasing predicted risks of DCS and complement depletion through the direct measurement of complement. We were unable to capture any clinical events of DCS and thus cannot comment on the role of complements in the development of clinical DCS. Predicted risk of DCS was determined by a probabilistic model of DCS thought to mirror soluble gas burden and relative bubble load. As the predicted risk for DCS and the theoretical bubble burden increased, there was a decrease in the amount of post-dive C3a. From these results we can theorize that the C3a component of complement is depleted as bubble burden increases. This supports the
results of others such as Pekna et al., who identified a decrease in the stable isomer of C3a iC3 (10). It is important to note that other markers of complement activation such as C4a and C5a were not affected. Only C3a appears to have been involved, suggesting that the alternate pathway was the means of complement depletion and the classic pathway was not affected.

Can complement be used as a surrogate marker for bubble burden and decompressive stress? The work of Bergh, et al., demonstrates that complement levels are highly fluctuant and unreliable over time (8). Our human data had a small to negligible coefficient of determination ($r^2$) and showed a great deal of individual variation in pre- and post-complement levels. Smaller studies have failed to detect any relationship at all (9). These findings suggest that complement activation is unreliable as an indicator of decompressive stress. However if more precise measurement of serum complement levels can be obtained, the potential exists for complement to be used as serological measure of estimated risk of DCS.

While the 1.3 ATA O$_2$ in helium study had a high rate of DCS, we were unable to obtain complete blood samples for a single case of DCS in our complement study. Because of this, it is difficult to comment on the role of complement in clinical DCS. This data however leaves open the possibility that complement activation may play a role in the development of clinical DCS. The rarity of actual DCS events makes this type of study design very difficult to implement. As high risk diving decreases with ROVs and deep diving suits replace divers, the actual incidence of DCS in the military and commercial arena will decline even further. This makes the possibility of future studies unlikely.

**CONCLUSION**

Decomplementation is associated with an increased risk of DCS in divers undergoing dives with high rates of DCS. There was a reduction in total Ca3 levels in divers after exposure to high risk dives. This decomplementation appeared to increase as the risk of DCS increased.

**ACKNOWLEDGMENTS**

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