Design, implementation, and assessment of an interactive simulation to teach undergraduate immunology students hemolytic disease of the newborn

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Abstract

Hemolytic disease of the newborn (HDN) is a potentially fatal condition caused by a Rhesus (Rh) antigen incompatibility between a mother and fetus. As a result, determining the Rh status of expectant parents is a routine clinical assessment. Both the physiological and immunological basis of this condition are taught to undergraduate students. At the University of South Australia, some undergraduate immunology students find this topic challenging. The author designed, implemented, and assessed the impact of an interactive simulation to facilitate student learning of HDN. The students were actively engaged in determining the blood grouping and Rh status of an expectant mother and father and then determining the possibility of developing HDN. The simulation was found to take only 15 min to complete yet led to a significant increase in student performance in an end of semester exam question. Student perceived understanding was found to significantly improve following the introduction of the simulation, even though the content had been covered in a formal lecture. Student feedback was highly positive of this learning approach. In conclusion, short, interactive simulations can be used effectively to enhance student learning of challenging concepts.

INTRODUCTION

Hemolytic disease of the newborn (HDN; Erythroblastis fetalis) is a potentially fatal condition caused by a Rhesus antigen incompatibility between a mother and fetus. In this condition, incompatibility between fetal and maternal Rhesus (Rh) antigen (Ag) expression on red blood cells (RBCs) leads to the priming of the maternal immune response (1–3). As a result, memory B cells are produced and reactivated on reexposure to Rh⁺ RBCs in future pregnancies. Transfer of even small numbers of RBCs can activate previously generated memory cells, leading to immunoglobulin class switching and IgG production. A unique characteristic of IgG is its ability to cross the placental barrier, which provides passive immunity to the fetus and newborn baby for up to 6 mo. However, in HDN, the IgG leads to Ab-mediated destruction of fetal RBCs through a combination of opsonization leading to activation of the classical complement pathway and phagocytosis of tagged RBCs. Depending on the timing of IgG production and the amount produced and resultant affinity, the impact on the fetus can range from mild to severe anemia, through to loss of the fetus (3). Clinically, the determination of potential HDN commences with blood typing the parents to determine their Rh status. If the mother is Rh⁻ and the father Rh⁺, then HDN can occur and needs to be addressed. Prevention of HDN is achieved postdelivery, through the injection of a mAb targeted to the Rh Ag [Rhogam, Rho(D)]. Elimination of fetal Rh⁺ RBCs from the maternal circulation prevents activation of the maternal immune system and thus the formation of memory cells. If this does not occur, the priming of the immune response leads to memory B-cell formation and the generation of a secondary immune response if the mother has another Rh⁺ fetus. Given the central role of the immune response in this disease state, students must understand all the immunological steps behind this disease process, its treatment, and why it is effective. Experience at UniSA has demonstrated that some undergraduate students find the initiation of HDN and the mechanism of treatment challenging to understand. The author has had prior success using interactive simulations to teach students complex biochemical and immunological concepts (4). As a result, an interactive simulation was developed to teach all aspects of HDN. This included determining the major blood group and Rh status of an expectant mother and father (5). The simulation then provided a detailed explanation of the disease process, outlining the immunological aspects as well as the mechanism of action of the treatment, Rhogam (6). Students could then test their understanding through 10 multiple-choice questions (MCQs). The impact of the simulation on student learning was assessed using a short answer question in the end of semester examination. Students that used the
Table 1. Tutorial questions used to teach the immunological principles of HDN

| Tutorial Questions | Ans: See introduction |
|--------------------|-----------------------|
| Qu 1: What is the basis of a Type II hypersensitivity reaction and how does it differ from a Type I? | Basis is a Type II hypersensitivity response which is initially IgM mediated and induces memory cells. On re-exposure, class switching leads to IgG production, which is placentally transferred and IgG-mediated effector mechanisms lead to RBC lysis. Differs from a Type I response as this is IgE mediated and hence the mechanism and effector reactions are also different. |
| Qu 2: How does HDN (Erthyroblastolis fetalis) develop, how can it be prevented and how does its treatment work? | |

Note, only 2 questions are included on this topic as the tutorial also covers Type I and II hypersensitivity reactions in one dedicated session; HDN, hemolytic disease of the newborn.

The simulation achieved significantly higher scores compared with those that did not access the simulation, with far fewer failing this question than those who did not access the simulation. Anonymous student feedback based on questionnaire responses identified strong agreement to using this approach and a significant improvement in student perceived understanding of the content. The use of a simulation allows for an interactive and educationally sound approach to teaching the principles of HDN to undergraduate students. This simulation is freely available to all teaching academics.

MATERIALS AND METHODS

Student Cohort

In 2018, 94 students were enrolled in Immunology (BIOL 2037) at UniSA. Students were predominantly enrolled in Laboratory Medicine (46%) and Medical Science (41%) programs, while the remainder were enrolled in Pharmaceutical Science (8.5%) or Science (1%) or were visiting overseas students (1%). All students were taught the principles of HDN in week 9 (of 13 wk) of the semester via a traditional lecture and accompanying tutorial. The students were expected to have attempted the tutorial questions (Table 1) before attending class, which covered the key immunological concepts (Table 2). The students were introduced to the simulation and actively encouraged to make use of it during their studies. The simulation was available for the remainder of the semester including the examination period.

Preparation of the Simulation

The simulation was prepared using Articulate Storyline (Version 2.13). An animated character gave both written and audio instructions to the user at each stage of the simulation (Figs. 1 and 2). This included an opportunity to determine the blood type of the parents and then determine the likelihood of HDN for future pregnancies. Text was converted to audio using a free text to speech website (http://www.fromtexttospeech.com). The simulation was packaged as a SCORM file and then loaded onto the course teaching page via the Moodle Learning Management System (LMS). The LMS analytics provided information including which students had attempted the simulation, when the simulation was used, the total time spent completing the simulation, the number of attempts, responses to the options in the simulation, and the score for the review MCQs.

Implementation of the Simulation

The rationale behind the development of the simulation was explained to students at lectures, tutorials, and via email communication in week 9 of the semester, immediately after the face-to-face lecture on HDN. Students were advised that the simulation should take ~15 min to complete. They were encouraged to complete the simulation to help revise the content and in preparation for the end of semester examination, which was scheduled ~6 wk later.

Analysis of Student Performance

Student performance in the end of semester examination was used to evaluate the effectiveness of the simulation. An HDN-specific question from the 2017 examination, when no simulation was available, was used in 2018 and the results compared following the inclusion of the simulation. Student performance was also analyzed within the 2018 cohort, comparing students who had or had not accessed the simulation (Fig. 6).

Student Feedback

On completion of the simulation, students were invited to complete a Likert scale questionnaire (Table 3). The questions related to their perceived knowledge, experience, and confidence in their understanding of HDN. Results were

Table 2. Key immunological concepts covered within the HDN simulation

| Key Immunological Concepts of HDN |
|-----------------------------------|
| 1: Impact of Rh⁻ vs. Rh⁺ immunological status. |
| 2: The immune system of an Rh⁻ individual will identify Rh⁺ RBCs as foreign Ags. |
| 3: The first exposure of an Rh⁺ immune system will lead to a primary immune response. |
| 4: Characteristics of a primary immune response include a lag phase, a low level of IgM, short-lived response, and memory cell formation. |
| 5: Re-exposure to Rh⁺ RBCs in a subsequent pregnancy leads to a secondary immune response. |
| 6: Characteristics of a secondary immune response include memory cell activation, reduced lag phase, high levels of IgG, which are long lived. |
| 7: IgG is the only Ig able to cross the placental barrier. |
| 8: IgG activates the classical complement pathway and effector cell-mediated hemolysis of Rh⁺ fetal RBCs. |
| 9: RBC destruction can lead to mild to severe effects. |
| 10: Treatment is a mAb injection directed against the Rh⁺ RBCs in the maternal circulation, which prevents immune system sensitization. |

Ags, antigens; Ig, immunoglobulin; Rh, Rhesus; RBC; red blood cells; HDN, hemolytic disease of the newborn.
The concepts are then further discussed in the following weeks tutorial session with relevant question(s) (Table 1), discussion and diagrammatic explanation. While HDN is addressed in the context of Type II hypersensitivity reactions, it encompasses fundamental immunological concepts central to all undergraduate immunology courses (Table 2). The simulation was hosted on the Immunology (BIOL 2037) course teaching homepage and made available immediately after the HDN lecture. The HDN-specific content was formally assessed in the end of semester examination, which also covered all other lecture and related practical material delivered during the semester. The student cohort was informed of the availability of the simulation and strongly encouraged to make use of it to enhance their learning and in preparation for the final examination, which was approximately 6 wk later. Analytics indicated that student usage of the simulation was limited immediately after the lecture; however, use peaked in the weeks leading up to the final examination. In 2018, most students accessed the simulation once, with some students engaging with the software twice. The simulation began with an animated character, Dr. Rita (Fig. 1), discussing the pregnancy status with a female patient, Jackie (Fig. 2). After providing a background on potential blood group incompatibilities, the requirement for blood grouping and Rh status is discussed. The student is then required to actively determine both the major blood group and Rh status of the mother and father (Fig. 3). At each interactive stage, immediate feedback was provided to
the student about the choice they had made. After determining that the mother is Rh−, and the father is Rh+, a virtual discussion is had around the potential of HDN (Fig. 4), which again reinforces the immunological basis of the disease as well as its treatment. Students can then test their understanding of HDN by answering 10 formative multiple-choice questions (Fig. 5).

**Student Performance**

In 2018, of the 94 students enrolled, 42 (45%) attempted the HDN simulation at least once during the semester. These students spent an average of 14.75 ± 1.1 min to complete the simulation. The mean score for the simulation-based MCQs was 53 ± 4.3 (±SE). There was a spectrum of scores for the questions, but it is not clear why there were such low responses in some cases. However, it does reinforce the observation that, for some students, this topic is challenging. In both 2017 and 2018, the question, “With the aid of a diagram, explain hemolytic disease of the newborn and how its treatment prevents the disease” was included in the end of semester examination. Performance in answering this question was then used as one measure of the impact of

**Table 3. Questions and mean student responses from the administered questionnaire, scored using a Likert-based scale**

| Question                                                                 | Likert Score (Mean) |
|--------------------------------------------------------------------------|---------------------|
| I found the simulation easy to use.                                      | 4.357/5 (5/9)       |
| The simulation enhanced my learning of the lecture material and made the concepts clear to me | 4.357/5 (6/7/1)     |
| I enjoyed this learning approach                                         | 4.143/5 (5/7/1/1)   |
| This approach should be used in other courses.                          | 4.286/5 (5/8/1)     |
| When I chose an incorrect answer, the feedback given assisted my learning. | 3.786/5 (2/5/6)     |
| The multiple-choice questions allowed to test my understanding of the material. | 4.429/5 (6/8)       |
| Before the lecture/simulation, my understanding of HDN was:             |                     |
| After the simulation, my understanding of HDN was:                      | 2.214/5 (0/5/4/2/3) |
| VG/G/N/L/NP                                                             |                     |

The values in parentheses are the number of responses for each of the ranking. SA, strongly agree (5 pt); A, agree (4 pt); N, neutral (3 pt); D, disagree (2 pt); VG, very good (5 pt); G, good (4 pt); L, low (3 pt); Low (2 pt); No prior knowledge (1 pt). HDN, hemolytic disease of the newborn. ***P < 0.0001, Student’s t test.
Figure 3. Students determine the major blood grouping of the mother and her Rhesus (Rh) status based on the hemagglutination pattern seen.

Figure 4. Summary of the physiological/immunological basis of hemolytic disease of the newborn (HDN). The mechanism of action of Rhogam is also discussed.
the simulation on student learning. The initial comparison was between the 2017 cohort, where no simulation was available to the 2018 cohort. When comparing the results from students that had accessed the simulation to the whole 2017 cohort, a statistically significant difference in student performance was observed (Fig. 6). In 2017, the mean score for the question was 1.7 ± 0.16 (out of 4) indicating that most student did not successfully pass this question. It is possible that this difference may have been due to variation between the two cohorts; however, there was no difference in overall performance when all students in each cohort were compared, suggesting no major difference in the cohorts (Fig. 6). The next analysis compared student performance for those who HAD versus HAD NOT accessed the simulation in the 2018 cohort. Consistent with the 2017 analysis, there was an even larger statistical difference in mean score between students that HAD (2.5 ± 0.17; means ± SE) versus those that HAD NOT (1.26 ± 0.18) used the simulation (Fig. 6). When individual student scores were compared, there were more students in the lower score range (0–2) in 2017, and for students who had not accessed the simulation. Conversely, more students were in the 2–4 score range if they had used the simulation, with more gaining full marks for the question (Fig. 6). These results suggested that there was indeed an impact of the simulation on student performance which was independent of any variation in student cohort.

Student Feedback

On completion of the simulation, students were invited to complete a questionnaire covering various aspects of their experience with the simulation. A total of 14 students (33%) responded to the questionnaire. A Likert scale was used, and scores applied to the responses for each question to facilitate analysis of the data. Most students found the simulation easy to use. This is important since any issues with navigating with learning technology are known to reduce student engagement (8). Also, the students strongly agreed that the simulation enhanced their learning of the lecture material and made the concepts clearer to them. Consistent with previous findings (4), the approach was found to be an enjoyable learning approach by most students. They also felt that this type of approach should be expanded in other courses. After the students had made a selection in the simulation, immediate feedback was provided. The student feedback relating to this question was the lowest for all questions, indicating that additional detail should be included. Not unexpectedly, the students appreciated the provision of formative MCQs to test their understanding of the material. When asked to self-assess their knowledge of the content, before the simulation, student understanding of HDN was perceived to be very low, even though the content had been covered in a lecture; however, after the simulation, there was a significant increase in student understanding.
perceived understanding of the content. While student perception does not always correlate with true student understanding, these results are consistent with the differences in performance seen in the final exam for this topic based on student usage.

Free Text Responses

In addition to the above questions, two free text questions were included. In response to the question “What were the best aspects of this approach, and how could it be improved,” the following written comments were received from six students. All comments were positive and again reinforced that students appreciated the use of a novel teaching approach as part of their learning:

1) “Interactive learning and outside the classroom, able to access anywhere, anytime. Makes filling the blanks for memory much better.”
2) “This teaching approach is great!”
3) “The quiz questions were good, and the background case study or story made the simulation engaging. I did not identify any area that is lacking, I enjoyed the sim, and it solidified my knowledge of the topic at hand!”
4) “The questions covered all aspects of the HDN problem in pregnancy and made me feel much more confident in being able to answer these questions correctly should they appear in the exam.”
5) “It guides me through the concept well.”
6) “Should provide a SIM for all the lectures.”

DISCUSSION

An interactive simulation that covered the immunological aspects of HDN was developed, implemented, and assessed. The simulation placed students in the role of a clinician involved in managing a patient. Students had an opportunity to determine the major blood group status of the mother and the father, as well as their Rh status. The Laboratory Medicine students learn this technique as part of their program enrolment; however, this is not the case for over half of the class. Thus exposure to the principles and graphical representation of results is a beneficial learning outcome (9). On determining that the mother is Rh−, and the father Rh+, the virtual clinician discussed the possibility of HDN, and the immunological basis is explained to the expectant mother. One area which some students find challenging relates to the mechanism by which the treatment, Rhogam mediates its protective effect. Given that the action of Rhogam is due to interfering with a primary immune response, this helps to consolidate, distinct, yet related immunological concepts. While the delivery of content is important, it is equally important that students can test their understanding before a formal assessment. As a result, 12 formative MCQs were included in the simulation. The mean score for these questions was a passing grade; however, it was perhaps lower than expected. This may have been due to the nature of the content or that the questions were only formative. It is likely that this score is lower than the true score as past experience and student feedback have indicated that some students purposely choose an incorrect answer to read the feedback that is provided. This feedback is a useful mechanism for students to identify different aspects of the content and thus broaden their understanding of the principles. If these questions were made as a summative component, perhaps a more accurate result would be obtained. Feedback on the impact of the simulation was judged using a Likert-based questionnaire. Feedback for all questions was found to be highly positive. Interestingly, student-based assessment of knowledge was found to significantly increase following use of the simulation. While it is acknowledged that students cannot accurately self-report (10), in many cases, overestimating their understanding, student performance in the exam, a more objective measure, was significantly increased in students that had used the simulation (2.5 ± 0.17) versus those that had not (1.26 ± 0.18). Importantly, this also demonstrates that a short (15 min), interactive exercise can significantly enhance student learning.

Based on past and current experience, interactive simulations have been useful in teaching advanced concepts of biochemistry, immunology, and writing skills and understanding (4, 11). The complexity of each simulation varies, as does the time commitment required for its production. However, a simulation can be generated by any academic willing to learn to use the software. An alternative approach would be to use an educational developer to generate the simulation, with the academic taking the role of content expert. There is a cost associated with this approach, but $2,000 U.S. dollars would be more than sufficient to generate a complete simulation of moderate complexity. In many cases, small institutional grants can be obtained to pay for this expense. Once generated, these simulations can be used...
for many years and any updates are relatively cheap to complete.

In relation to the questionnaire, most responses were highly positive. However, one aspect that required some attention relates to the immediate feedback provided for the interactive elements of the simulation. This question scored the lowest, so additional detail will be included so that a more global answer is provided to the students. It is acknowledged that students appreciate sufficient feedback if they choose an incorrect answer (12).

While this simulation was aimed at undergraduate immunology students, it can be used to teach students in related allied health areas, for example, sonography and midwifery. Indeed, the author has used the simulation as one component to teach interprofessional learning to nursing, laboratory medicine, and sonography students (manuscript in preparation).

In conclusion, an engaging and interactive simulation was developed and found to significantly enhance student understanding of the fundamental concepts relating to HDN. Simulations can be effectively used to teach complex concepts and are an excellent aid for traditional methods of teaching. They can also be used offline, when students cannot physically attend campus, as has and is occurring throughout COVID. The simulation discussed in this manuscript is freely available to any staff member that would like to assess its impact on their teaching. Please contact the author for additional information.

ACKNOWLEDGMENTS

The author thanks Hayley Timms for help with Articulate Storyline and Rita Costabile for testing of the simulation and critical review of the manuscript.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

M.C. conceived and designed research; M.C. performed experiments; M.C. analyzed data; M.C. interpreted results of experiments; M.C. prepared figures; M.C. drafted manuscript; M.C. edited and revised manuscript; M.C. approved final version of manuscript.

REFERENCES

1. Avent ND, Reid ME. The Rh blood group system: a review. Blood 95: 375–387, 2000 [Erratum in Blood 95: 2197, 2000]. doi:10.1182/blood-V95.2.375.
2. Moise KJ. Hemolytic disease of the fetus and newborn. Clin Adv Hematol Oncol 11: 664–666, 2013.
3. Visser GH, Di Renzo GC, Spitalnik SL, FIGO Committee Safe Motherhood and Newborn Health. The continuing burden of Rh disease 50 years after the introduction of anti-Rh(D) immunoglobulin prophylaxis: call to action. Am J Obstet Gynecol 221: 227.e1–227.e4, 2019. doi:10.1016/j.ajog.2019.05.019.
4. Costabile M, Timms H. Developing an online simulation to teach enzyme kinetics to undergraduate biochemistry students: an academic and educational designer perspective. In: Evidence-Based Faculty Development Through the Scholarship of Teaching and Learning (SoTL), edited by Plews RC, Amos ML. Hersey, PA: IGI Global, 2020, p. 281–302.
5. Quinn JG, Tansey EA, Johnson CD, Roe SM, Montgomery LE. Blood: tests used to assess the physiological and immunological properties of blood. Adv Physiol Educ 40: 165–175, 2016. doi:10.1152/advan.00079.2015.
6. Mittendorf R, Williams MA. Rho(D) immunoglobulin (RhoGAM): how it came into being. Obstet Gynecol 77: 301–303, 1991. doi:10.1097/00006250-199102000-00029.
7. Sullivan GM, Artino AR Jr. Analyzing and interpreting data from likert-type scales. J Grad Med Educ 5: 541–542, 2013. doi:10.4300/JGME-5-4-18.
8. Bourgonjon J, Valcke M, Soetaert R, Schellens T. Students’ perceptions about the use of video games in the classroom. Comp Educ 54: 1145–1156, 2010. doi:10.1016/j.compedu.2009.10.022.
9. Maldarelli GA, Hartmann EM, Cummings PJ, Horner RD, Obom KM, Shingles R, Pearlman RS. Virtual lab demonstrations improve students’ mastery of basic biology laboratory techniques. J Microbiol Biol Educ 10: 51–57, 2009. doi:10.1128/jmbe.v10i99.
10. Ross JA. The reliability, validity, and utility of self-assessment. Pract Assess Res Eval 11: 1–13, 2006. doi:10.7275/9wpv-vv65.
11. O’Flaherty J, Costabile M. Using a science simulation-based learning experience to develop students’ active learning, self-confidence and critical thinking in academic writing. Nurse Ed Pract 47, 2020. doi:10.116/j.nepr.2020.102839.
12. Chin C, Osborne J. Students’ questions: a potential resource for teaching and learning science. Studies Sci Ed 44: 1–39, 2008. doi:10.1080/03057260701828101.