As the war began, Maryland hovered on the brink of secession. It was important to the national government to keep the State in the Union, or Washington would be surrounded by enemy territory and completely cut off from the Northern States. The influence of Northern sympathizers, abetted by the presence of Federal troops during most of the war, kept the State in the Union. But this did not prevent the Confederacy from recruiting many volunteers in Maryland. Prominent among them were Colonel Bradley T. Johnson, commander of the First Maryland Regiment, C.S.A., and Admirals Franklin Buchanan and Raphael Semmes who served in the Confederate Navy.

In September 1862, General Lee invaded Maryland and at South Mountain and Antietam fought General McClellan's army in two of the most bitter battles of the war. Both sides suffered heavy casualties. McClellan permitted Lee to retreat across the Potomac without further molestation. It was while "Stonewall" Jackson was leading his troops through Frederick on the way to Antietam that the Barbara Fritchie incident—subject of the famous poem of John Greenleaf Whittier—is said to have occurred. On the political front, President Lincoln seized upon this as the proper moment for issuing the Emancipation Proclamation.

The following summer, Lee again advanced into Maryland. This time, he continued into Pennsylvania and at Gettysburg between July 1 and 3 engaged General Meade in a bloody struggle which left Lee's army so badly battered that he had no choice but to retire into Virginia.

In July 1864, Lee, hoping to divert some of Grant's troops from the siege of Petersburg, sent General Jubal Early to raid Western Maryland and threaten Washington. General Early defeated the Union Forces at the Battle of Monocacy on July 9 and advanced to the outskirts of Washington. At the Battle of Fort Stevens, which Lincoln witnessed on July 11, the Union lines held. Grant, meanwhile, sent fresh troops from Virginia forcing Early to retire. It was during this third invasion of Maryland that large indemnities were levied on Hagerstown and Frederick.

The war's end had its tragic aftermath for Maryland. John Wilkes Booth, a native of Harford County, assassinated Lincoln on April 14, 1865, and made his escape through Southern Maryland into Virginia. During this episode, Dr. Samuel A. Mudd, a Charles County physician, set Booth's broken leg for which he was accused of assisting in the assassination plot and sentenced to prison.