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Macrophages from disease resistant B2 haplotype chickens activate T lymphocytes more effectively than macrophages from disease susceptible B19 birds

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A B S T R A C T

Resistance to respiratory pathogens, including coronavirus-induced infection and clinical illness in chickens has been correlated with the B (MHC) complex and differential ex vivo macrophage responses.

In the current study, in vitro T lymphocyte activation measured by IFNγ release was significantly higher in B2 versus B19 haplotypes. AIV infection of macrophages was required to activate T lymphocytes and prior in vivo exposure of chickens to NP AIV plasmid enhanced responses to infected macrophages. This study suggests that the demonstrated T lymphocyte activation is in part due to antigen presentation by the macrophages as well as cytokine release by the infected macrophages, with B2 haplotypes showing stronger activation. These responses were present both in CD4 and CD8 T lymphocytes. In contrast, T lymphocytes stimulated by ConA showed greater IFNγ release of B19 haplotype cells, further indicating the greater responses in B2 haplotypes to infection is due to macrophages, but not T cells. In summary, resistance of B2 haplotype chickens appears to be directly linked to a more vigorous innate immune response and the role macrophages play in activating adaptive immunity.

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1. Introduction

Disease resistance to a variety of pathogens has been associated with MHC (major histocompatibility complex) haplotypes in several species, including mice and chickens (Banat et al., 2013; Briles and Briles, 1982; Dunnington et al., 1992; Heinzelmann et al., 1981; Joiner et al., 2007; Kim et al., 2008; Briles and Briles, 1987; Lamont, 1998; Mays et al., 2005; Mills, 2015; Yoo and Sheldon, 1992).

In the chicken, MHC B haplotypes have been shown to display differential resistance to several viruses; including Marek's disease virus, avian leukosis virus, Newcastle disease virus, Rous sarcoma virus, infectious bronchitis virus (IBV) and avian influenza virus (AIV), as well as Salmonella (Banat et al., 2013; Briles and Briles, 1982; Lambrecht et al., 2004; Dunnington et al., 1992; Heinzelmann et al., 1981; Joiner et al., 2007; Kim et al., 2008; Lamont, 1998; Mays et al., 2005; Yoo and Sheldon, 1992).

Following infection with IBV, birds with the homozygous B2 haplotype have been shown to be more resistant to respiratory illness than B19 haplotype birds (Banat et al., 2013). Furthermore, B19 monocytes from peripheral blood mononuclear cells (PBMC) were slower to differentiate into macrophages upon in vitro culture than monocytes from the PBMC of birds with the B2 homozygous haplotype (Dawes et al., 2014). Macrophages, important cells of innate immunity, are directly involved in cellular interactions with pathogens, resulting in release of cytokines that activate other immune cells and in antigen presentation to cells responsible for adaptive immunity (Medzhitov and Janeway, 1997, 2000a,b; Romagnani, 1992). Dawes et al. (2014) demonstrated that macrophages isolated from B2 birds were significantly more responsive than B19 derived macrophages to polyriboinosinic acid-polyribocytidylic acid (poly I:C), which simulates viral RNA replication and to IFNγ, an indicator of lymphocyte activation.

Considering the ease of activating B2 macrophages compared with the B19 macrophages, it was of interest to compare their...
relative capacity to stimulate T lymphocytes and what conditions, such as prior exposure to antigen, are essential for activation. In our previous studies, using infected homozgyous kidney cells as antigen presenting cells, in vivo vaccination of vectors expressing the NP of AIV were strong inducers of MHC restricted CD8 T lymphocytes (Singh et al., 2010a,b).

The current study compares the roles of macrophages in the activation of homologous and heterologous T lymphocytes. Unlike kidney cells, macrophages are considered professional antigen presenting cells, expressing both MHC I and MHC II and thus are capable of activating CD8 and CD4 T lymphocytes, respectively. AIV infection in chicken macrophages has been demonstrated previously (Xing et al., 2010; Barjesteh et al., 2014). Therefore, macrophages infected in vitro with AIV were ideal in evaluating differences in ex vivo activation of T lymphocytes purified from B2 and B19 birds vaccinated with the AIV NP.

2. Materials and methods

2.1. Experimental animals

Animal procedures were approved and conducted according to guidelines established by the Western University of Health Sciences, Pomona, California (Western University) Institutional Animal Care and Use Committee. Fertilized eggs (from either B2/B2 or B19/B19 haplotype birds), acquired from the laboratory of Dr. W. Elwood Briles at Northern Illinois University, were incubated and hatched under standard conditions at Western University (38 °C and 50–65% humidity). Post hatch, chicks were held in an incubator maintained at 38 °C and 50–65% humidity for 24 h, before transferring to a brooder, pre-heated to 35 °C, in the vivarium of the University Research Center at Western University. In addition to daily health monitoring, fresh food and water were provided ad libitum. Room temperature was adjusted to and maintained at 32 °C until 3 weeks of age when chicks were transferred to open cages in the vivarium of the uninfected chickens incubated with freeze thaw supernatant (Singh et al., 2010a). After infection by real time PCR. Primer sequences: CDNA synthesis: TGCTCTCGAATGCAAGCT, PCR primers: GAATCCTGGGAATGCT- GAAA and GTGCTCTGATTCTTGGAGT. PCR conditions were as follows: 95 °C for 10 min-hot start, 40 cycles of 95 °C for 15 s, 60 °C for 30 s according to manufacturer instructions for the Biotool 2x Sybr Green qPCR Mix (Biotool, Houston, Tx). Viral infections were also confirmed with TCID50 as previously described, using cytopathic effects (CPE) and the hemagglutination (HA) assay (Moresco et al., 2010; Reed and Muench, 1938). Briefly, macrophages were isolated as described above and 500 µl of PBMC in media were placed in 48 well plates at 5 × 10^5 cells/ml. Stocks of 10^2 EID50 of virus were used to infect wells at 1:10 dilutions in duplicates and CPE was evaluated by microscopy. Plates were frozen at –80 °C for 24 h before thawing, when supernatants were used for HA assays (RBC from uninfected chickens incubated with freeze thaw supernatant (Moresco et al., 2010) and TCID50 determined.

2.5. T lymphocyte preparation

The T lymphocytes were prepared from 3 ml of peripheral blood diluted in an equal volume of PBS from the interphase after Ficoll-histopaque density (1.077) gradient centrifugation (Pei et al., 2003; Seo and Collisson, 1997; Singh et al., 2010a). After collecting cells from the interface and washing with PBS, mononuclear cells were suspended in 3 ml of RPMI 1640, supplemented with 10% FBS (Invitrogen, La Jolla, CA) and incubated for at least 45 min in tissue culture plates to allow for macrophage attachment. After removing the unattached cell population, the B lymphocyte population was depleted using an AIV equilibrated nylon wool column, as previously described (Seo and Collisson, 1997; Singh et al., 2010a). The T lymphocyte enriched preparations were washed twice and suspended in RPMI before use.

2.6. Ex vivo activation of T lymphocytes

T lymphocytes, purified from PBMC, were incubated with or without macrophages as described for stimulation with AIV infected kidney cells (Seo and Collisson, 1997; Singh et al., 2010a). After removing the media from each well when macrophages were 70–80% confluent (approximately 5 × 10^5 cells per well of a 24 well plate), T lymphocytes were added at a concentration of 5 × 10^6 in 500 µl per well of RPMI 1640 with 10% FBS. The infected or uninfected control macrophages were incubated with T lymphocytes for
24–48 h before quantifying the amount of IFNγ produced in each well as the indicator of T lymphocyte activation. Studies included the co-culture of 1. Heterologous, NP vaccinated T lymphocytes and AIV infected macrophages. 2. Homologous T lymphocytes from birds that were not vaccinated with NP with infected macrophages and 3. NP vaccinated T lymphocytes with homologous, uninfected macrophages.

2.7. Detection of activated T Lymphocytes

Activation of T lymphocytes was evaluated by determining the concentration of IFNγ in the clarified supernatants from cultured T lymphocytes using a commercial ELISA specific for ch-IFNγ (Invitrogen, La Jolla, CA, Pei et al., 2003; Singh et al., 2010a, b). Nitric oxide secretions induced in the cultured macrophage and T lymphocyte combinations were quantified using a Griess reagent assay according to the manufacturer’s protocol (Sigma-Aldrich, St. Louis, MO) and described by Singh et al. (2010a, Dawes et al., 2014). The concentration of nitrite produced was determined using sodium nitrite solutions with concentrations of 1–20 μM as standards. The concentration of any non-specific production of nitric oxide by soluble factors was adjusted by subtracting the nitrite concentration of supernatants from macrophages cultured without T lymphocytes from the supernatants of the macrophages cultured with T lymphocytes.

2.8. Interactions in the absence of macrophage-T lymphocyte, cell-to-cell interactions

Supernatant fluids collected from infected or uninfected macrophages were placed on homologous T lymphocytes collected as described above. After 24 h of exposure, the supernatant fluids from these T lymphocytes were collected and clarified by centrifuging at 1500 RPM for 10 min before quantifying IFNγ in the supernatant using ELISA as described above.

2.9. Depletion of CD4 and CD8 T lymphocytes

T lymphocytes were purified before incubating with mouse, anti-chicken CD8 antibody as described by Singh et al. (2010b). The CD8 and CD4 T lymphocytes were separated by antibody-mediated depletion using Dynabeads (Invitrogen, La Jolla, CA; Singh et al., 2010a). T lymphocytes were labeled with either mouse anti-chicken CD8 or mouse anti-chicken CD4 monoclonal antibodies (MAb) (Southern Biotech, Birmingham, AL) at a concentration of 1 μg/10^6 cells in PBS containing 0.1% bovine serum albumin fraction V (Sigma-Aldrich, St. Louis, MO) before incubating at 4 °C for 30 min. The cells were washed twice with PBS to remove unattached antibodies before incubating with rat anti-mouse IgG coated Dynabeads, DynaMag-2 (Invitrogen, La Jolla, CA) according to manufacturer’s instructions. Unlabeled cells in the supernatants were collected before confirming the purity of CD4 or CD8 depleted T lymphocyte preparation by FACS analysis using fluorescent labeling with CD4 or CD8 MAb (Bohls et al., 2006). The purity of CD4 or CD8 T lymphocyte subpopulations after antibody depletion of either CD8 or CD4, respectively, was 95%.

2.10. ConA stimulation of T lymphocytes

T lymphocytes, purified as described above, were incubated in 48 well microtiter plates at a concentration of 5 × 10^5 cells/ml with 250 μl per well. 2.5–5 μg of ConA per ml (Sigma) were added to each well before incubating for 16–24 hr, when the supernatants were removed and the concentration of IFNγ was determined using ELISA. Sample readings were equivalent after either 16 hr or 24 h and whether using 2.5 or 5 μg/ml of ConA.

2.11. Statistical analyses

The GraphPad Prism 6 (GraphPad Software Inc, La Jolla, CA) was used for statistical analyses. Statistical tests performed were unpaired t-tests, non-parametric, (Mann Whitney test or Kruskall Wallis test) with statistical significance considered at p < 0.05. The data is represented in figures as averages with standard errors.

3. Results

3.1. In vitro AIV infection of macrophages

Chicken macrophages are readily infected with AIV (Lyon and Hinshaw, 1991; Xing et al., 2010; Barjesteh et al., 2014). Therefore, in order to compare the potential for macrophages from B2 or B19 defined birds to activate T lymphocytes, chicken macrophages were infected with the low pathogenic H5N9 strain (A/Turkey/Wis/68) of AIV (Singh et al., 2010a). Infection was detected by immunofluorescent staining of preparations of macrophages derived from birds of either haplotype (Fig. 1). TCID50 was determined by CPE and HA assays to be 10^{2.2}/500 μl, with no detectable difference for either

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Fig. 1. Infection of macrophages with low pathogenic H5N9 AIV (A/Turkey/Wis/68). Infection of cell culture differentiated B2 or B19 derived macrophages with AIV. 10× objective was used for all microscopy pictures. (A) Brightfield macroscopy of AIV infected B2 macrophages, (B) IFA stained uninfected B2 macrophages and (C) IFA stained AIV infected B2/B2 macrophages. (D) Brightfield macroscopy of AIV infected B19 macrophages, (E) IFA stained uninfected B19 macrophages and (F) IFA stained AIV infected B19 macrophages.
haplotype. Viral RNA detection in B2 and B19 macrophages 45 min (after viral uptake) and 16 h incubation after infection indicated that there were no significant differences between B2 and B19 haplotypes \((n = 3\) uninfected controls/haplotype, \(n = 5\) infected/haplotype). Viral replication calculated with the delta ct method against viral load at the 45 min infection time point was 38 fold more in infected B2 macrophages and 34 fold more in infected B19 macrophages, showing comparable replication. These results demonstrate that there were no significant differences \((p > 0.05)\) in viral replication and infectivity in B2 and B19 haplotype macrophages.

3.2. \(B2\) derived AIV infected macrophages are better at activating \(NP\) primed homologous \(T\) lymphocytes than \(B19\) derived infected macrophages

We have previously shown that the NP of AIV expressed in cDNA or an adenovirus vector is an exceptional CD8 T lymphocyte antigen (Singh et al., 2010a, b). Therefore, chicks vaccinated with the NP cDNA were used as a source of influenza primed T lymphocytes. The potential for \(B2/B2\) or \(B19/B19\) macrophages to stimulate \(NP\) primed T lymphocytes was examined using AIV infected macrophages cultured with homologous T lymphocytes purified from NP vaccinated birds (Fig. 2A).

Activation of macrophages at 3, 5, 10, 16, 21 and 35 days post-vaccination with the NP cDNA, was measured by quantitation of NO, reflective of a response to stimuli including cytokines such as IFN\(\gamma\) secreted by the co-cultured T lymphocytes (Fig. 2A). Macrophages from both haplotypes show activation, however, stimulation of \(B2\) macrophages was considerably greater at any day post-vaccination than that of the \(B19\) macrophage cultures. Infected macrophages alone in the absence of T lymphocytes produced negligible NO (Fig. 2B), indicating the stimulation of macrophages and consequent NO release observed was due to co-culture and activation of T cells.

Activation of T lymphocytes from 5 to 26 days post-vaccination was evaluated using ch-IFN\(\gamma\) ELISA (Fig. 2C). The differences seen between the haplotypes were even more pronounced than measuring NO release from macrophages. There was essentially no detectable activation of \(B19\) T lymphocytes by homologous infected macrophages on days 10 and 16 after NP vaccination. The \(B2\) derived primed T lymphocytes were consistently more potently activated by infected macrophages than \(B19\) derived infected T cells from the influenza NP vaccinated birds.

3.3. AIV infection of macrophages was required for \(T\) lymphocyte activation

\(T\) lymphocytes, collected at varying times between 3 and 42 days after vaccination of birds with the NP cDNA, were cultured with uninfected macrophages. Macrophages that were not infected, regardless of the haplotype, were incapable of activating \(T\) lymphocytes as evidenced by lack of detectable IFN\(\gamma\) in the ELISA (data not shown).

3.4. Prior exposure to NP is not required for stimulation of \(T\) lymphocytes by infected macrophages

Macrophage stimulation did not require that the source of T lymphocytes be from chickens vaccinated with the NP cDNA. Naive T cells from either \(B2\) or \(B19\) unvaccinated chicks were stimulated with homologous infected macrophages resulting in IFN\(\gamma\) release from T cells. However, the levels of stimulation were lower when compared with T cells derived from NP vaccinated chicks. In coculture experiments of infected macrophages and naive T-cells, \(B2\) macrophages released more nitric oxide than \(B19\) cells (Fig. 3A). Whereas IFN\(\gamma\) release of naive \(B2\) T cells could be detected when co-cultured with AIV infected \(B2\) macrophages, \(B19\) infected macrophage mediated stimulation of \(B19\) naive T cells was again significantly less as determined by the IFN\(\gamma\) ELISA (Fig. 3B).

3.5. \(T\) lymphocyte activation by infected macrophages does not require cell-to-cell interaction

Antigen presentation implies direct interaction between the macrophage and T cell. In order to examine the necessity for cell-to-cell interaction, T lymphocytes were cultured with supernatants collected from infected macrophages rather than with the infected macrophages. Just as \(B19\) infected macrophages had little influence on \(B19\) T lymphocytes in the previous experiments, the supernatants from the \(B19\) infected macrophages had no impact on the \(B19\)
T lymphocytes. In the absence of cell-to-cell interaction, supernatants from B2 infected macrophages did stimulate homologous T lymphocytes (Fig. 4A). However, the magnitude of IFN\(\gamma\) released was significantly less compared to cells that were co-cultured and allowed cell-cell interaction between T lymphocytes and infected macrophages (Fig. 4B), indicating that antigen presentation plays an important role in this activation.

### 3.6. Infected B2 macrophages activate IFN\(\gamma\) production from B19 T lymphocytes

In order to determine requirements for MHC compatibility between the infected macrophage and the T lymphocyte, MHC cross-matched experiments between AIV infected macrophages and T lymphocytes were performed. Infected B2 macrophages were able to stimulate B19 T cells (Fig. 5). In contrast, B19 macrophages did not stimulate B2 T lymphocytes, further confirming that the B19 macrophages are poorly stimulated by AIV infection themselves and consequently not activating the T cells via cytokines.

### 3.7. Both CD4 and CD8 T lymphocytes are activated by infected macrophages

In order to determine the T cell source of activation, either CD8 or CD4 T lymphocytes from vaccinated B2 birds were purified prior to exposure to AIV infected homologous macrophages (Fig. 6). Both CD4 and CD8 depleted T lymphocytes, respectively, were activated in the presence of infected macrophages. Therefore, both T cell phenotypes from B2 vaccinated chicks responded similarly to homologous AIV infected macrophages with no significant difference. In the absence of infected macrophages neither CD4 nor CD8 control T lymphocytes were stimulated.

### 3.8. T cells from B19 haplotype chicks were better responders to nonspecific ConA stimulation

To assess whether the T cells of either B2 or B19 haplotypes have

![Fig. 3. Infected B2 macrophages stimulated homologous T cells from unvaccinated chicks. Production of NO by macrophages co-cultured with T lymphocytes (A). Activation of T lymphocytes was determined by detection of IFN\(\gamma\) (B). The differences between the B2 and B19 macrophage stimulation not exposed to the NP cDNA vaccine were \(p < 0.001\) for both figures A and B.](image)

![Fig. 4. Supernatants from B2 infected macrophages stimulated B2 T lymphocytes to produce IFN\(\gamma\). Supernatants from infected macrophages were used to stimulate homologous T lymphocytes from vaccinated (A) or unvaccinated (B) birds. Differences in both figures were \(p < 0.01\).](image)

![Fig. 5. Stimulation of T lymphocytes by infected B2 macrophages was not entirely MHC restricted. The B2 infected macrophages stimulated heterologous B19 T lymphocytes while B19 infected macrophages had essentially no impact on B2 T lymphocytes (\(p < 0.05\)). B2 uninf M or B19 uninf M represent uninfected macrophages cultured with heterologous /B19 or /B2 T cells from vaccinated birds. B2 AIV M represent infected B2 derived macrophages cultured with B19 derived T lymphocytes and B19 AIV M represent infected B19 macrophages cultured with B2 derived T lymphocytes.](image)
B19 infected macrophages were poor at stimulating T lymphocytes. Load and infectivity being comparable in both haplotypes. Thus, the potential for non-specific activation by a lymphocyte mitogen in the absence of macrophages, both T lymphocytes from either B2 or B19 birds were exposed to ConA. Surprisingly, while both types of lymphocytes responded to ConA, the B19 T lymphocytes responded significantly better than the B2 derived T lymphocytes (Fig. 7). Differences in the activation of the T lymphocytes thus could not be attributed generally to a lack of B19 lymphocyte function.

### 4. Discussion

These studies demonstrate dramatic functional differences in the impact of the MHC B2 and B19 haplotype defined macrophages on adaptive immunity, specifically, activation of T lymphocytes. Quantification of macrophage responses co-cultured with T cells consistently demonstrated higher nitric oxide responses by the B2 haplotypes. In addition, activation of IFNγ responses from either homologous B2 or even B19 derived T lymphocytes co-cultured with AIV infected macrophages were demonstrated, despite viral load and infectivity being comparable in both haplotypes. Thus, the B19 infected macrophages were poor at stimulating T lymphocytes.

The AIV specific responses of T cells have been described after inoculation of birds with an AIV cDNA expressing the NP (Singh et al., 2010). The NP cDNA had been previously shown to encode a more effective T lymphocyte antigen than even the AIV hemagglutinin protein. Although B2 derived T lymphocytes from birds that had never been exposed to AIV NP also responded to homologous infected macrophages, prior exposure of the B2 birds inoculated with NP cDNA consistently resulted in significantly greater responses to AIV infected B2 derived macrophages.

It was of interest to determine if T cell stimulation required cell-to-cell interaction with the infected macrophage or if supernatants from macrophages alone activate lymphocyte production of IFNγ. T cells incubated with supernatants from B2 AIV infected macrophages in the absence of macrophages were able to stimulate T cells to release IFNγ. However, we also found that the amount of IFNγ released was lower than in experiments with T cell-macrophage contact.

B2 infected macrophages could stimulate IFNγ from heterologous B19 T lymphocytes, most likely by release of cytokines, as antigen presentation does not play a role in the MHC mismatched cell culture. However, the homologous cultures consistently resulted in greater induction of B2 T lymphocyte IFNγ compared to the heterologous cultures, indicating that although cytokines released by the infected macrophages play a role in the stimulation of T cells, antigen presentation with direct cell-cell contact has a significant effect on this activation. Another indication of a feedback activation in this co-culture system is the increase in NO levels released by B2 macrophages co-cultured with T lymphocytes. This NO release is likely caused by release of IFNγ by the T cells after activation, which has been previously shown to be the major activator of macrophages, or possibly by other cytokines that stimulate the nitric oxide release.

The previous studies by Singh et al. (2010) indicated that the CD8 rather than CD4 T cells were induced ex vivo with homologous AIV infected kidney cell, that is, the nonprofessional cell cultures. Infected macrophages in the current study induced both CD4 and CD8 T cells, as indicated by the release of IFNγ by both CD4 and CD8 depleted T cells. Since macrophages, as professional antigen presenting cells, are active players for both innate and adaptive immunity, they would be expected to be more comprehensive at alerting the T lymphocyte population of infection. Whereas kidney cells express MHC I, macrophages express both MHC class I and MHC class II antigens and thus have the potential to activate CD8 and CD4 T cells, respectively (Burgdorf and Kurts, 2008). In addition, the release of cytokines by infected macrophages would equally affect CD4 and CD8 positive T lymphocytes, although the release of IFNγ was too great to be just mediated by cytokines alone.

Detection of macrophage activation using nitric oxide measurements by co-culturing with T lymphocytes is complicated by the fact that the macrophages are infected with AIV. Therefore, it was critical that direct determination of IFNγ confirm the macrophage and T lymphocyte interactions and that in the absence of infection, macrophages did not stimulate T cells to produce IFNγ or other cytokines that might in turn activate the macrophages to release nitric oxide.

Cytokine production, specifically secretion of pro-inflammatory molecules, has also been associated with increased resistance against disease in chickens (Ferro et al., 2004; Swaggerty et al., 2004). Dawes et al. (2014) showed that, in response to poly I:C or IFNγ stimuli, responses of macrophages derived from the more resistant B2 chickens, were dramatically greater than the responses of macrophages from the more IBV susceptible B19 haplotype chickens.

The demonstration of differences early in infection described by Banat et al. (2013) had suggested that innate immunity initially contributed to the enhanced resistance of the B2/B2 birds to IBV
associated clinical illness. In the current study, we used AIV rather than IBV because macrophages are readily infected with AIV. Although belonging to different viral families with distinctly different properties, both viruses have single-stranded RNA genomes and both have enveloped virion particles. It should be assumed that IBV, although not readily infecting macrophages, infects epithelial cells and consequential pathology would be expected to activate innate immunity.

The current studies suggest that the B19/B19 derived macrophages are deficient in their response to external stimuli, that is, macrophage infection. However, the deficiency is not universal for all immune cells from the B19 haplotypes, since B19 T lymphocytes surprisingly not only respond to ConA stimulation, but responded significantly better than the B2 derived T lymphocytes. Upon stimulation of macrophages with ch-iFNa, irizyary and Drechsler (personal observations) also have now found differences in a number of cellular pathways in macrophages from either B19 or B2 birds.

The current studies, focusing on T lymphocyte responses, confirm and highlight the importance of the interphase in the chicken between macrophages and adaptive, specifically T lymphocyte, immunity. These studies strongly suggest that macrophage function rather than T lymphocyte function, is the driving factor in the differences observed between B2 versus B19 activation of T lymphocytes. Differences in macrophage function, correlating with differential innate immune responses (Dawes et al., 2014), corroborate recent studies that demonstrate a much more important role macrophages play, in general, in directing the adaptive immunity, particularly T cell responses, in a variety of diseases including cancer and autoimmunity (Mills et al., 2000; Mosser, 2003; Mills, 2012; Biswas et al., 2012).

5. Conclusions

B haplotype chicken lines provide an excellent resource for studying the genetic bases of MHC associated disease resistance and susceptibility, and the relationship between macrophage function and disease resistance. Further studies elucidating molecular mechanisms of this activation are needed in order to understand the underlying genetic causes.

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